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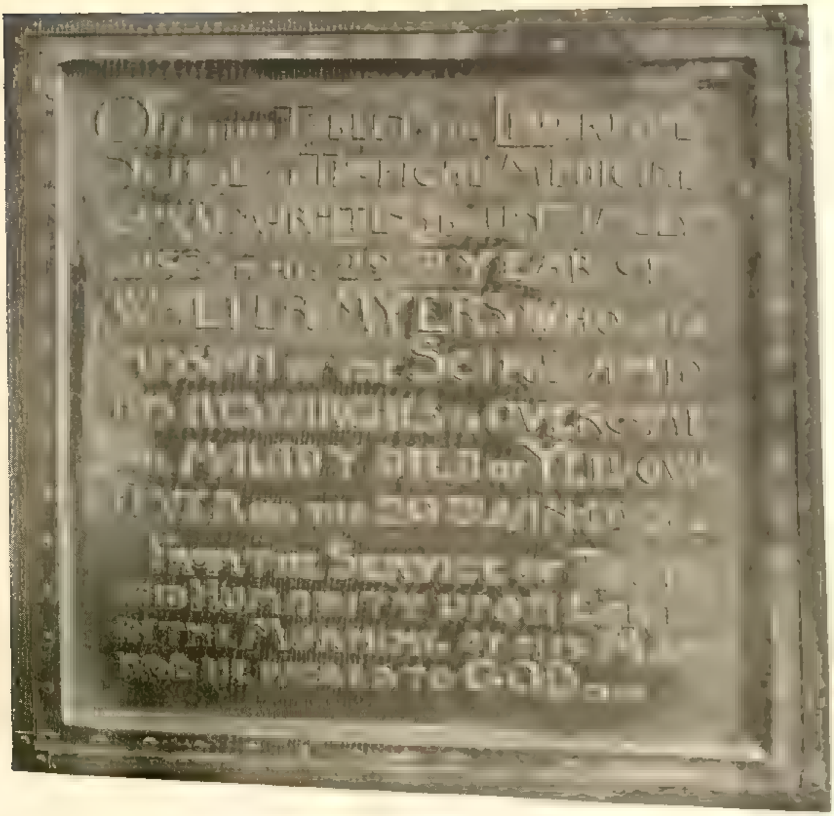

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INTRODUCTION

The Liverpool School of Tropical Medicine despatched the fifteenth expedition comprising Dr. Anton Breinl and the writer to Manáos in April, 1905, in order to investigate yellow fever and the diseases of the Amazon State. This expedition was the second sent to the Amazon region, the first one in 1900 having been stationed in Pará. Shortly after its arrival in Manáos, both members were attacked by yellow fever, and subsequent complications in the case of Dr. Breinl unfortunately necessitated his return to Europe, and thus his valuable services were lost to the expedition.

No trained assistance was procurable and all the work has had to be performed single-handed. The report presented embraces only a portion of the work of the research laboratory in Manáos. I have to thank those gentlemen who were kind enough to contribute chapters to this report; it would have been impossible otherwise to have dealt with such a variety of subjects. It is hoped to complete later the account of all the investigations pursued during the years 1905 to 1909, and to publish, at short intervals, a series of papers on subjects affecting the welfare and hygienic conditions of the Amazon region. The district is so rich in material that it would require a large staff of workers to carry out the researches on the spot; by collecting the material and forwarding it to Europe, it is possible to induce European authorities to issue short monographs on their subjects.

In many respects the 1905-1909 Expedition to the Amazon differs from the expeditions previously despatched by the School. A permanent laboratory was necessary, and attention had to be given to a multiplicity of details which are usually foreign to a travelling commission. On the other hand numerous advantages arose out of the permanency of the laboratory, since patients could be kept under continued observation and experiments inaugurated which would have been impossible under other conditions.

The main object of the expedition was to investigate yellow fever, and, if possible, endeavour to advance our knowledge of this disease. Chimpanzees have been successfully inoculated; rabbits and guinea-pigs have exhibited certain reactions when inoculated with infective blood from yellow fever cases or subjected to the bites of infected *Stegomyia calopus*. The results at first were very erratic, but, subsequently, with improvement in technique and important details, the reactions were more positive and could be gauged with certainty. Unfortunately it has proved impossible to test the reactions by human inoculations, and the chimpanzees were too few in number to be able to prove all the different points. Many tests have to be made and experimental work revised at all points, and therefore it is considered inadvisable to make public the exact technique employed until an exhaustive report can be presented. The experiments were discontinued for lack of animals susceptible to the disease, and the expedition returned to England in February, 1909. A new expedition has now been organised and provision made for an adequate supply of chimpanzees. With improved accommodation for the experimental animals, and with the experience gained from the work of the past years, it is confidently expected that an early and satisfactory report can be published.

An expedition should endeavour to assist the local authorities and aid the medical profession. Therefore the resources of the laboratory in Manáos were placed at the disposal of the State, the medical fraternity, the hospitals, and the poor of the city. Examinations of blood, agglutination reactions for typhoid and paratyphoid, bacteriological examinations of water and milk, post-mortems and pathological reports were made, and the laboratory so conducted as to be of the greatest possible service to the community. As a consequence much routine work had to be performed. From the manifestations of sympathy and the co-operation extended to the expedition by the State authorities and the doctors of Manáos, it is believed that the work of the laboratory has proved of real service.

It is a matter of congratulation that the relations between the State and myself were of the most cordial character. Every facility was offered by those in authority to lighten the task of the investigation; laboratory accommodation was provided, and instructions given that the aims of the expedition should be expedited in every way.

The investigation was begun during the term of office of the Governor Coronel Constantino Nery, and was continued under the Acting-Governor Coronel Affonso de Carvalho, both of whom displayed much interest and were of great assistance to the expedition. The present Governor of the State of Amazonas, His Excellency Coronel Antonio Bittencourt, manifested much sympathy with the work, and extended an invitation to the expedition to return to Manáos to complete the various researches which were left unfinished.

The Directors of the Servico Sanitario, Dr. Alfredo da Matta and Dr. Miranda Leão, and their colleagues on the medical board, did everything that was possible to promote the work of the laboratory. The active and loyal co-operation of the medical profession in Manáos also contributed largely to the success of the expedition. Without this aid, freely rendered on all sides, little work could have been accomplished, and the intimate relationship which existed between all parties has been attended with pleasant memories. It is impossible to specify individually the names of our co-workers, since it would involve the enumeration of the whole of the medical profession in Manáos; but to one and all I wish to tender my warmest thanks.

In this expression of gratitude mention must be made of the authorities of the Santa Casa de Misericordia, and Portugueza Beneficente, and the Sisters of the Religious Orders who are responsible for the nursing of the patients. Here again help was freely and continuously given.

The business firms and their staffs in Manáos, and the subscribers to the expedition have been of assistance in every possible way. Invidious though it be to single out an individual firm, I must, however, express my warm appreciation of the interest which the Booth Steamship Company exhibited in the work. The sub-committee in charge of the organisation of the expedition was composed of two of the Directors of the Company, and no request, however extravagant and exacting it might seem, was refused. Without their co-operation experimental work with such costly animals would have been impossible, nor could the chimpanzees have been imported under mosquito-proof conditions.

In compiling this report, which is the outcome of nearly three and a half years of actual observation, I have endeavoured to be as open and fair-minded as possible. The suggestions have been brought forward solely with the view to assist the authorities in Manáos in their efforts to improve the local sanitary conditions. I can testify to the great improvements that have been made in the sanitation of Manáos since my arrival in 1905. Commercial enterprises are rapidly advancing, and as commerce extends the population of the Amazon region will steadily increase. Modern sanitary methods and anti-mosquito measures will therefore become a necessity even in the smaller towns along the Amazon and its tributary rivers.

In conclusion, my thanks are due to Sir Rubert Boyce for the use of his department in the Thompson-Yates Laboratories, and to Mr. Newstead for his advice and help. It has been a matter of much regret to me that serious ocular troubles should have prevented the completion of the detailed study of the material designed for this report. It is hoped, however, to publish the results of the experimental work at an early date.

H. W. T.

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THE SANITARY CONDITIONS AND DISEASES PREVAILING IN MANÁOS, NORTH BRAZIL, 1905-1909, WITH PLAN OF MANÁOS AND CHART

BY

H. WOLFERSTAN THOMAS, M.D., C.M. (McGILL),

Expedition to the Amazon, 1905-1909

(*Fifteenth Expedition of the Liverpool School of Tropical Medicine.*)

Manáos, the capital of the State of Amazonas, was selected as the headquarters of the Expedition. It is the second largest city in the Amazon district and is one of the most important cities in North Brazil.

The city is situated on the Rio Negro, a few miles above its confluence with the River Amazon, and lies in latitude 3° 08′ 30″ and longitude 59° 59′ 27″ west of Greenwich; its height above the sea level is only about 3·40 metres. It is 900 miles' river journey from the city of Pará at the mouth of the Amazon river and 1,300 miles from the town of Iquitos, Peru. The traffic is entirely carried on by ocean and river steamers of up to 6,000 tons, plying between Hamburg, Liverpool, New York and Manáos.

The population of Manáos is about 60,000, comprising some 33,000 intra-urban and 28,000 extra-urban inhabitants. The greater number are Amazonenses (persons born in the Amazon State), but many Brazilians from other States, especially Cearenses, have settled in the city. There are about 4,000 to 5,000 foreigners, chiefly Portuguese, Italians and Spaniards, with some hundreds of Americans, English, French, Germans, Syrians and Turks.

It is impossible to give accurate figures, as there is a large floating population. Manáos is the commercial centre of the vast rubber trade of the State. The port is used by all the river steamers, and is the arrival and departure point for all those connected with the rubber trade of the interior. The following tables, compiled from figures by Dr. Alfredo da Matta,* give an idea of the foreign and national passenger movement in the Port of Manáos.

FOREIGNERS ARRIVING AND DEPARTING

	Arrivals			Departures		
Year	From interior	From exterior	Total	For interior	For exterior	Total
1901 ...	1,208	2,953	4,161	483	3,189	3,672
1902 ...	1,294	3,165	4,459	589	3,189	3,778
1903 ...	1,230	3,074	4,304	476	3,340	3,816
1904 ...	560	1,660	2,220	243	2,066	2,309
1905 ...	763	4,911	5,674	385	3,563	3,948

BRAZILIANS ARRIVING AND DEPARTING

	Arrivals			Departures		
Year	From interior	From exterior	Total	For interior	For exterior	Total
1901 ...	13,647	18,766	32,413	12,872	13,534	26,406
1902 ...	12,011	11,859	23,870	10,371	9,768	20,139
1903 ...	16,131	13,375	29,506	7,749	10,597	18,346
1904 ...	9,634	15,610	25,244	7,959	7,609	15,568
1905 ...	17,435	21,310	38,745	11,104	13,069	14,173

Climatology.

Complete observations are difficult to obtain, but the following table, compiled by Dr. Lopes de Campos† for the year 1903, gives a fair idea of the conditions in Manáos.

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* Dr. Alfredo da Matta. Paludismo, Variola, Tuberculose em Manáos. Revista Medical de S. Paulo, Nos. 14 and 15, 1908.

† Dr. Lopes de Campos. Climatologia Medica do Estado do Amazonas Imprensa Official. Manáos, 1903.

Month	Rainfall in millimetres	Number of days on which rain fell	Relative humidity	Velocity of wind in metres per second
January ...	214	23	78	1·77
February ...	201·2	19	79	2·06
March ...	262	19	78	1·88
April ...	155	15	76·9	1·75
May ...	116	20	78	1·62
June ...	23	8	67·9	1·68
July ...	30·6	7	67·9	1·60
August ...	17·4	4	65·7	1·83
September ...	65·8	10	68·5	2·13
October ...	57·	8	63·9	2·43
November ...	69·4	14	70·2	1·89
December ...	184	22	73·4	1·79

The temperature, while not exceptionally high, is very constant. The hot months extend from September to January, the highest temperature occurring in October. The months of April and May are slightly cooler. During the rainy season a fall of two to four degrees centigrade occurs about daybreak, so that the early hours of the day are quite refreshing. The thermometer averages about 24·5° C. at 6 a.m., at which time there is generally a light N.E. breeze; at noon, a slight breeze may spring up for a short time.

MAXIMUM TEMPERATURES (CENTIGRADE) OF THE HOT MONTHS*

Year	September	October	November	December
1901 ...	32·7	32·9	32·6	31·9
1902 ...	35·8	37·5	36·6	35·0
1903 ...	35·6	36·6	36·0	35·0
1904 ...	35·1	37·2	36·4	36·4
1905 ...	36·0	36·4	35·0	35·0

AVERAGE TEMPERATURES

Year	Mean	Minimum	Maximum
1901 ...	27·3	24·3	37·4
1902 ...	27·9	20·7	34·3
1903 ...	28·5	22·0	36·0
1904 ...	28·8	21·8	32·7
1905 ...	28·8	22·2	36·4

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* Dr. Alfredo da Matta, *loc. cit.*

A curious phenomenon is the cold spell of three days (*dias de friagem*) which may occur in the month of June. It is usually preceded by some heavy rains, mists and squalls. A very cold southerly wind occurs, accompanied by a rapid fall of temperature, reaching 16° C. in a few hours. In 1907 the *friagem* occurred in the first week in July, the temperature falling to 18° C.

The topography of Manáos is well seen on the accompanying plan. From the river bank the ground gradually rises towards the North, where a watershed causes the formation of two igarapés (*creeks*), the Igarapé da Cachoeira Grande on the West and the Igarapé da Cachoeirinha on the East. The city is intersected from North to South by the Igarapés Manáos and Bittencourt, while a small one, the Igarapé da Castelhana on the eastern side empties into the Igarapé da Cachoeira Grande. The Igarapés Cachoeira Grande and Cachoeirinha form very large collections of water at all times of the year.

The rise and fall of the Rio Negro is an important factor, there being a difference of 50 to 60 feet between highest and lowest river (Chart). The river is at its height in June, and then gradually recedes until the end of October. A slight rise may occur in November and be followed by a further slight fall. It then remains stationary until January, when it commences to rise. At low river, in consequence, the upper reaches of the smaller Igarapés Manáos, Bittencourt, etc., are dried up and, as the water recedes, many puddles and holes containing water are left to furnish breeding places for mosquitos At high river, the water from the Rio Negro has backed up in the igarapés, and vast areas of low lying land are completely submerged The offices of many of the chief commercial firms and banks being situated along the river front, an extremely high river causes much inconvenience and damage, as the cellars and low-lying sections of the streets become flooded. Since this condition persists for several weeks, the propagation of mosquitos and disease is favoured by these collections of stagnant water. The effective functioning of the low-level sewers is hindered, but this latter annoyance will be abolished by the new sewage lifting-pumps.

Streets. The general plan of the city shows many broad avenues and streets running North and South, and in places a number of squares. In the city proper the main streets are laid with granite blocks, a few, however, having asphalt blocks; the less important streets are generally laid with rubble and macadam. The sidewalks are composed of stone slabs or artificial pavement, with gutters of stone.

Electric light is employed to illuminate the streets of the city and many of the houses and offices. The residents are fortunate in being able to use electric fans, as they are almost indispensable in cooling the rooms and driving away mosquitos.

The system of electric trams has proved a great boon to Manáos. The lines to Flores and other suburban parts are well patronised in the evenings by the residents for the cool ride through the swampy areas.

Disposal of refuse. Collection of house refuse is made nightly by scavengers, who give an efficient service to the residents of the centre of the city.

Drainage. There is a system of sewers for the intra-urban population, but none at all for the extra-urban inhabitants.

Water supply. The water supply is derived from the Rio Negro,* the inlet pipes being well above the city and sufficiently far from the shore to ensure a supply of uncontaminated river water. In 1909, a most elaborate filtration plant of automatic compressed air polarite filters was installed. The filtered water is pumped up to the two reservoirs, Mocò and Castelhana, which are built on the heights of

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* The following analysis by Mr. W. J. Debdin, F.I.C., F.C.S., of samples of the Rio Negro water is reproduced by the courtesy of the Manáos Improvements Co., Ltd., from a report prepared by Messrs. Beesley, Son & Nichols, M.Inst.C.E.

Appearance	Odour at 100° Fahr.	Total Solids		Phosphoric Acid	Hardness		Ammonia		Chlorine Grains per Gallon	Oxygen absorbed from Permanganate at 80° Fahr.		Organic Elements			Nitrogen as Nitrates, &c. Grains per Gallon	Cultivation on Gelatine Plates Colonies per c.c.	Micro-Filter mm. per litre	Pathogenic Organisms	Microscopical Examination
		Grains per Gallon	Appearance on Ignition		Total	Permanent	Free	Albuminoid		In 15 mins.	In 4 hrs.	Carbon	Nitrogen	Total					
					Degrees		Grains per Gallon			Grains per Gallon		Parts per 100,000							
Brown colour with suspended particles	None	3·2	Blackened	Very slight trace	1·4	1·2	·0040	·0125	0·81	·566	·950	·800	·060	·860	0	684	11·0 Poisonous Metals Nil Strongly Plumbo-Solvent	Bacillus Coli Communis in 0·1 c.c. Streptococci not detected	Oxide of Iron and Peaty Matter. Numerous dead Aquatic Organisms. Daphina, &c. [illegible] Moving Organisms, Infusoria, Algæ, &c.

Manáos. The water is then conveyed to the houses of the inhabitants by gravitation through a system of pipes.

Both the drainage and water systems are being greatly improved and extended by the Manáos Improvements Company, Limited. A modern system of sewers, house connections, and sewage disposal is almost completed, and, with the new water supply, the sanitation of the city will be greatly improved. In so many tropical towns the great evils are the lack of a pipe-borne water supply and an efficient drainage system; the absence of these necessities naturally helping to propagate disease. With the completion of such important works, many of the present sanitary evils which prevail in Manáos should vanish.

Food supply. The cost of living is very great, the prices of actual necessities being absurdly high as compared with other tropical countries. The beef is of average quality, but the supply is limited. Cattle-raising is not taken up very extensively, as only certain parts of the State are suitable for ranches, but the Rio Branco and parts of the country along the Rio Amazonas are well adapted and furnish a fair number of cattle. Under such circumstances the poorer class of citizens in Manáos are rarely able to afford fresh beef, and their diet consists chiefly of dried beans, farinha, goat flesh, salt cod and some dried local fish, plantains, fruit. Fowls, ducks, eggs, etc., are all dear, the demand exceeding the supply. In consequence, large quantities are imported from South Brazil and the lower reaches of the Amazon district.

Fresh-water turtle and various kinds of fish are excellent and, during low river, are both cheap and abundant.

Many delicious and nourishing fruits grow wild in the State, but even the commoner ones fetch high prices, as the natives do not make any serious attempts to cultivate them. Green vegetables, such as lettuce and other salad greens, tomatoes, and radishes can be grown in Manáos, and this occupation affords employment to a large number of Portuguese market gardeners. We consider the State should encourage the people by exempting them from certain forms of taxation, so that greater quantities of produce could be grown and retailed at prices within the means of the indigent class. Fresh green vegetables are an absolute necessity, and should be within the reach of the poor as well as the rich. Such vegetables as potatoes, cabbages, etc., have to be imported from Europe, and therefore are accounted luxuries. The supply of fresh milk is limited, as all milking cows have to be imported, and it is chiefly used by invalids and rich residents. Practically all the milk is boiled before being consumed.

Artificial ice of excellent quality is manufactured. The more universal employment of refrigerators would be encouraged if the price of ice was reduced.

The slaughter-houses along the Igarapé da Cachoeira Grande and the central markets in the town are of recent construction and admirably serve their purpose. The sale of meat, fish and turtle, etc., is under medical inspection, and an efficient supervision is maintained.

The Santa Casa de Misericordia is the sole public hospital in Manáos for the reception of indigent patients. The institution was designed for about 160 beds, but this accommodation is inadequate, as the hospital has to care for the numerous patients arriving from the interior of the State. The wards are overcrowded for many months in the year, but despite such difficulties the patients are well attended to. Numerous improvements have been instituted, and a fully equipped operating theatre, outdoor and maternity departments, and a separate barrack for tuberculous cases, enhance the value of this hospital. The institution is managed by a lay committee who appoint a large staff of the local medical men, assisted by the Sisters of the Order of Santa Anna, to attend to the various departments. The medical work of the expedition was carried on in this hospital, and after an associateship of three and a half years, we can warmly testify to its excellence.

The Sociedade de Beneficiencia Portugueza conducts a well-planned hospital for the needs of the Portuguese colony. Mosquito bars are furnished for all the patients, and wire gauze screened observation and isolation wards are provided for the treatment of their yellow fever cases.

A small isolation hospital well removed from the city is used for small-pox patients and a few indigent lepers. A State lunatic asylum is in course of erection.

Another small hospital is maintained by the Federal authorities for the treatment of the Federal troops.

An admirable institution is provided by the State for the education of orphan girls. It is known as the Institut Benjamin Constant, and is thoroughly equipped on modern lines.

In former years the authorities inaugurated many extensive works which involved the raising of the levels of the low lying areas, the filling up of some of the igarapés, and the making of extensive excavations. Unfortunately, as is often the case, mature consideration does not appear to have been exercised before undertaking the improvements. Igarapés were incompletely filled in; streets were made across swamps, thus damming back the waters; and street levels altered without a thought as to the consequences. The surface waters draining into these places created miniature lakes and swamps where, formerly, only puddles had existed. In 1897 and 1898 such works were in progress in all parts of the city, and with the excavating, filling and re-excavating which seems to have occurred, collections of water were multiplied all over Manáos, mosquitos bred in their thousands, and fevers reigned supreme. Hundreds of workmen were attacked with malaria, and the deaths from this disease alone rose to 1,074 in 1898 and to 1,495 in the year 1900. Happily for Manáos and her citizens such a condition of affairs is at an end, and the authorities are alive to the necessity of carrying out improvements methodically and with the collaboration and advice of the sanitary authorities.

During our residence in Manáos, 1905 to 1909, we have noted the efforts of the various departments in the direction of abolishing the many foci of water which had resulted from the past operations, and we desire to record our appreciation of the immense difficulties which have been successfully overcome. It is hard for a newcomer to realise how much has been accomplished unless he learns from old survey maps, etc., that where many of the buildings and streets are, at one time there was nothing but igarapés and swamps.

The situation of Manáos admits of many improvements being effected. There still exist too many low lying areas of land in the very midst of the populated districts, which are either swampy throughout the year or, at any rate, during the rainy season. These swamps all serve as breeding places for Anophelines, Stegomyia, Culex, etc. The propagation of flies is also encouraged by the populace being afforded opportunities for the depositing of refuse which, in many instances, has been found to be of a most objectionable and insanitary nature.

Many of the streets are on a much higher level than the adjoining land. The market gardeners use the sloping land for the growing of vegetables; some of these gardens are situated in the heart of the city, a most objectionable feature, as the rain-water barrels and cisterns used by the gardeners for the storage of water are usually unscreened, and have often been found full of mosquito larvae.

The municipality has filled up many of these low-lying areas and heads of creeks (igarapés) with the soil obtained from the excavations of new streets and the cutting down of the high banks of earth in various parts of the town.

It is impossible to accomplish much with the means at their disposal. The soil has to be transported in small carts, so that the whole operation is very costly and tedious. A cheaper and far quicker method is by excavating with the steam shovel, combined with the use of a light railway and gravel cars as employed by the Manáos Harbour Company in the filling in of their works. This is well exemplified in the case of the new Alfandega, which is built entirely on filled-in land.

The general plan of the laying out of the streets in the new part of Manáos, in the upper residential sections and in the outskirts of the city, allows of wide streets, with abundance of air space.

Manáos resembles the majority of cities in which the old or original sections of the settlement consist of narrow streets and confined areas. In the old quarters and business section of the town the streets are narrow and the buildings closely packed together. All the available space has been built upon, and no thought has been given to ventilation or illumination. Many of the small shops and, in some cases, the offices of business firms are so crowded alongside and around one another as to preclude adequate ventilation and illumination. There is generally a back-yard adjoining, but it is small, and too often encumbered with barrels of water or empty boxes. In some yards wooden structures have been erected contrary to the by-laws of the municipality.

The type of house commonly seen in Manáos is of Portuguese architecture, and ill-adapted for a tropical climate. A small dwelling generally consists of a front room facing the street, an inner chamber which is usually a bedroom and opens into the dining room; leading from this is a passage along which are a couple of bedrooms overlooking a little yard; a dark kitchen is at the end of the passage, and immediately adjacent to the kitchen, the latrine and shower bath. The inner rooms receive air and light only from the outer ones, while the sleeping quarters along the passage are lighted and ventilated from the yard.

Such cramped, ill-lighted and badly ventilated houses are usually occupied by five to eight persons. The air space is quite inadequate, and even the houses of the wealthy citizens are so packed together and planned as to prevent through ventilation.

The residents appear to have only one object in view, to live as near to the business section as possible, despite the exorbitant price of land and high rentals. Very few villas exist, and even these are largely constructed on the same lines as the smaller dwellings, dark inner rooms being universal. Bungalows with broad verandahs, and with rooms so adapted that a current of air can continually circulate, are regrettably rare. Owing to lack of space these can only be erected at some distance from the business quarter, but the tram service is so good that there is no reason why such residences should not be built.

The working classes, such as the Portuguese and Italian labourers, are often far more badly housed. They tend to gravitate to the midst of the city, and overcrowding is very common. On some streets are to be found low one-storied barracks subdivided so as to accommodate ten to twenty, and even forty people. Here the overcrowding is extremely bad, three and four people living in a low room, 3 metres by 4 metres, lighted and ventilated only by a wooden shutter and door, which are generally kept closed. The sanitary conveniences in such places consist of a latrine which is supposed to be used by all the tenants, but this is not always the case. The footpath in the yard is laid with ill-fitting stone flags, the rest of the narrow yard being unpaved and littered with uncovered barrels of water. The odour of urine and smell of human excrement is nauseating.

Sometimes these places are situated adjoining a market garden, as on Rua Dr. Moreira (Plan near 25) and other streets of the city. Some of the tenants are washerwomen, who must perforce do their washing here. Human beings herded together in such an area can only breed filth and disease. In one of these places (Plan 25) the garden slopes down to the swampy land, which is only partially filled in and from which we have collected many Anophelines (p. 20). The native in the outskirts of the city, in his mud hut with thatched roof, fares better than do these people. It should be remembered that many of the tenants of these places are newly arrived immigrants. Can it be surprising that they should suffer from yellow fever, etc ?

Other tenements of a better class are scattered over the city. These have larger yards, and overcrowding is not marked. The sanitary arrangements are better, but are usually insufficient for the number living in the restricted area, while the habits of many of the tenants conduce to unhealthy surroundings.

Underground cellars are rarely seen, but many of the houses have low, ill-lighted basements which the residents adapt for kitchen and servant quarters. The authorities are adverse to such practice and try to prevent them being used as sleeping-quarters. The floors of a great many houses are only raised a half to one metre from the ground, ventilation being provided by a small iron-screened opening on the street. The screening is either too open or the bars are broken so that cats, rats and other vermin can enter. When the houses are sufficiently raised from the ground the inhabitants frequently litter the space under the flooring with old boxes and other rubbish. Such places are insanitary and favour the breeding of vermin which would be a serious matter in the case of an outbreak of plague.

THE BREEDING PLACES OF MOSQUITOS

In the City Proper.

Stegomyia calopus is to be found all over the populated districts of the city. It is not an exaggeration to state that 98 per cent. of all dwelling houses and places of business harbour, in the building or in the yard, foci of water which give the female *Stegomyia calopus* a place where she can lay her eggs and where the young larvae can hatch out.

One of the ordinances of the city is that all cisterns used for the storage of water must be screened with fine wire gauze. This ordinance is not always obeyed, and very often the screening is most defective (usually around the ball and cock of the inlet water pipe of the cistern). The sanitary authorities summon householders for not having the cisterns screened, but too often is it the case that in a month or so afterwards the screen is taken off or propped up.

It is the custom of the householder to store his empty bottles in the yard. These are usually laid on the side and piled up at such angle that rain water is collected and another ideal breeding place for the mosquito is provided. Empty tin cans, buckets, earthenware pots, etc., adorn the back yard, nearly all being so placed as to collect water. Very often these are only cleared away after an inspection has been made by the authorities, to be followed shortly by another collection of useless rubbish.

Barrels for rain-water are unscreened or covered with a loose-fitting top which more often than not is left off. Gutter and gutter pipes may be broken or blocked. All these, if defective, collect water and facilitate the propagation of the mosquito. Empty tins, bottles, even old baths, are often stored under the flooring and, when the floors are washed, water will drip through the cracks in the flooring and collect in the cans, etc. The usual method of washing a one-storied house is to throw a couple of bucketsful of water on the floor and then rub the boards with brooms and sluice on more water. We have seen the workmen, during building operations, too lazy to clear away the old tin cans and similar rubbish on the ground before nailing down the floor. (One house badly infested with mosquitos was a source of much worry to us until we took up a few boards and found some old saucepans partially filled with water and teeming with larvae.)

Many yards laid with cement have depressions or cracks in which water may lodge. The man-hole of the drain pipe in the yard for carrying off the rain-water may be so constructed as to allow of residual water to the depth of 1·5 to 2·0 cm. In some houses which have cellars underneath, we have observed collections of water on the floor, due to the splashing of heavy rain on the sidewalk, again resulting in the breeding of mosquitos.

Some window-sills and the accompanying rain pipe are so constructed as to harbour an astonishing amount of water if the small drip-pipe gets choked. Rain has come down the chimney and remained in the rusty stove, and larvae have accordingly flourished.

In houses which have been vacant for some time one is almost sure to find evidences of larvae in the water of the closet pan.

Usually these houses have improperly screened or unscreened cisterns, and the cellars and back yards are often littered with a collection of rubbish which favours mosquito propagation.

In workshops the water-trough of the grindstone, the tub by the forge, and, in offices, even the water receptacle used for wetting the brush for the copying book have been found to harbour eggs or larvae.

Builders also too frequently neglect to screen the barrels used for storing water for mortar making, etc. In the digging of foundations for buildings the soil is heaped up and puddles formed. On the iron girders which are left lying about for a long time, rain-water can collect in the depressions.

In some of the street gully-pipes, at the man-holes leading to the sewers, the mouth of the upright pipe is fully 3·5 cm. or more above the surface of the cement. Here month after month quantities of Stegomyia larvae are found breeding.

The municipality is responsible for the existence of many fountains in the city which again are simply breeding places for mosquitos.

Swamps and Igarapés.

Numerous swamps* and igarapés are to be found in the city proper, and the inhabitants in these localities suffer from the effects of malaria.

The very extensive swamp in the valley at the back of Ruas Cearense, Ferreira Penna and Commendador Clementino (Plan 5), is responsible for the presence of much malaria and anaemia among the residents on both sides of the valley. In 1908 a partial filling was attempted, but up to my departure, in January, 1909, no improvement was observable. The sides of the valley are much contaminated with refuse. Many women use the place for washing clothes.

The igarapé crossing Rua Occidental behind Rua Monsenhor Coutinho (Plan 4) is also a prolific breeding ground.

The igarapé at the back of Rua Luiz Antony and extending up to Rua 10 de Julho (Plan 2) is a source of much disease. Much washing of clothes is done here, and the breeding of mosquitos is further helped by the barrels sunk by the washerwomen in the soil for the purpose of obtaining clean water. There are a very considerable number of these receptacles, and many Anopheline larvae have been obtained from them throughout the year.

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* Newstead, R., and Thomas, H. W.; The mosquitos of the Amazon Region. Annals of Tropical Medicine and Parasitology, Vol. IV, No. 1, p. 141-142, 1910.

The igarapé at the back of Rua Independencia (Plan 1), the head of which is now filled in by the Rua da Codeia, is still a breeding ground for mosquitos by reason of the dank vegetation along the sides. The back yards of many of the houses of Rua Independencia are flooded when the river is high. Up to 1908 there existed a large and deep body of water just east of Rua Codeia, which occurred from the incomplete filling up of this arm of the igarapé. Countless hoards of Stegomyia, Culex and Anopheline larvae bred there. Year after year cases of malaria and yellow fever occurred amongst the inmates of the neighbouring houses and hotels. By the complete filling in of this comparatively small area the authorities have destroyed one of the most dangerous breeding spots in Manáos.

A most prolific breeding spot is the low land near Rua dos Andrades along the projected Avenida 13 de Maio (Plan 25), where collections of water harbour many *Mansonia titillans* and *Culex fatigans* larvae. *Stegomyia calopus* abound in the houses of the neighbourhood. Some of the old houses are built on piles immediately over this foul-smelling water. During the rainy season, the back yards of many of the houses in the vicinity are flooded and the neighbourhood pestered by swarms of these mosquitos. Partial filling has been accomplished, and it is to be hoped that this will cease to be a danger spot.

The swamps along the proposed Avenida 13 de Maio, from Rua Candido Marinho up to and on the northern side of Rua 10 de Julho (Plan 6) are sources of propagation for both Anophelines and Culicines. The swamps on both sides of Rua 10 de Julho have contained, at all times of the year, larvae of *Stegomyia calopus*, *Cellia albimana*, *Uranotaenia geometrica*, *Culex fatigans*, and *Mansonia titillans*. Immediately adjacent to these swamps is the Hospital de Beneficencia Portugueza (Plan 7), and in its corridors we have captured from time to time Anophelines, *Uranotaenia*, etc. Not far removed from the other side of this hospital are other swamps from the Igarapé Manáos, all contributing their quota of Anophelines. As previously mentioned this hospital possesses several screened wards for yellow fever cases, but only mosquito nets for patients suffering from other diseases, and, in the course of the year, many cases of malaria are under treatment in the wards.

Around the Igarapés Manáos and Bittencourt, larvae of *Cellia argyrotarsis* and *Ce. albimana* are always to be found. During the period of falling river many small pools and puddles are left which breed numbers of these Anophelines and swarms of *Culex fatigans* and *Mansonia titillans*.

At very low river the upper reaches of these igarapés are comparatively dry, but foci of Anophelines can nearly always be discovered. With rising or high river the larvae of *Cellia* are to be found along the edges among the dank vegetation clothing the sides of these igarapés.

Along the numerous arms of the Igarapé da Cachoeirinha (Plan 10, 15, 16) occur many breeding spots for Anophelines. The land in many places is low-lying and swampy for the greater part of the year, and only becomes dry at very low river. The breeding places for mosquitos are augmented very considerably by the number of pigs allowed to roam. These animals are always rooting about in the swampy land, and create numerous holes which retain water for a long time, and serve as favourite spots for the Anopheline ova and young. Along the ravines and swampy lands the inhabitants, in order to obtain clean water for drinking and washing purposes, sink small tubs and barrels into the sandy soil, and these have always been found to contain many imagines of malaria-conveying mosquitos. The occupants of the houses adjacent to the swamps and arms of this igarapé naturally do not escape malaria, as is evidenced by the very large number of admissions to the Santa Casa and Benificente Portuguese Hospitals. During the rainy season it is the exception to find these people free from the parasite. On the eastern side (Plan 26) of the igarapé the inhabitants of the houses along the bank and at the Colonia Oliveria Machado (Plan 24) all exhibit signs of the ravages of malaria.

In the North, which is the highest part of Manáos, the igarapés, swamps and ravines, as can be seen from the map, are fairly extensive. Villa Municipal (Plan 19) is situated in this section, and many Anophelines are bred in the swamps at the sides and back of the racecourse, near the Wireless Station.

Around the Plano Inclinado (Plan 3) are many spots for the propagation of *Cellia albimana*, caused by the numerous indentations of the Rio Negro and the Igarapé da Cachoeira Grande. Malaria exacts a heavy toll from the residents of this district.

The suburbs of São Raymundo (Plan 23) and Colonia Oliveira Machado (Plan 24), on the far sides of the Igarapés Cachoeira Grande and Cachoeirinha, are on fairly high ground, but many ravines and swampy low-lying areas exist. The majority of the houses are situated along and in these areas.

Further outside the limits of the city, very extensive swamps occur. Unfortunately these districts are thickly populated by the poorer class, with a few residents of the better class. Near the old pumping station of the waterworks (Plan 21), the Bosque (Plan 22), Pensador (Plan 27) and Flores are a series of swamps which exist all the year round, and during the rainy season cover very extensive areas. From every one of these swamps larvae of *Cellia albimana*, *Cellia argyrotarsis*, *Culex fatigans*, *Mansonia titillans*, and *Uranotaenia geometrica* have been taken, and adult specimens captured in the houses of the inhabitants at the Bosque, Pensador and Flores. Numerous *Stegomyia calopus* have been found in the larger houses where rain-tubs and barrels are kept unscreened and full of water.

The Bosque, Pensador and Flores are prolific breeding places for Anophelines, and the residents in these localities suffer from malaria. The residents of these districts are responsible for many of the breeding-places, as they are too indifferent to fill up any of the shallow pools or to dig small trenches to drain the natural hollows. In these localities (Plan 22) much sand occurs which is used by the local builders; as a consequence extensive sandpits exist, which, during the rainy season, are full of water, and serve as breeding-places for both Culicines and Anophelines. Such foci occur all along the swamps and near to the houses. It is therefore not astonishing to find so many cases of malaria in proportion to the number of the inhabitants.

We fully realise the desire of the authorities to stamp out malaria in the confines of Manáos, and are aware of what has already been or is to be done.

Much good work can be accomplished by attacking the upper reaches of the Igarapés Bittencourt, Manáos, Tocos, S. Vicente and Bica. The filling of these igarapés must be gradual, as their extent is so great; but when accomplished valuable areas of land will be available for building sites. Then again it would be advantageous if the upper reaches of the igarapés were filled in *to above* the level of ordinary high river. At the same time the banks and beds of these igarapés should be cleared of all the small trees, undergrowth and dank vegetation which grow so luxuriantly along their entire course. All the washing pools used by the washerwomen should be either done away with or removed to one particular area, which will allow of their being inspected and kept free of receptacles which help to harbour mosquitos and their larvae. The pigs should be prevented from roaming about the areas of the igarapé and the necessary measures enacted.

Further, a small brigade of men should be appointed to discharge or fill the small pools left by the receding waters, draining the hollows where possible and only oiling spots which are absolutely impossible to fill or drain.

Rubbish should not be allowed to be dumped at these igarapés unless of material suitable for filling, and then only at those places which are being filled.

After the initial cleaning and stripping of the trees, brushwood, and high vegetation from the banks and bed of the Igarapés Bittencourt and Manáos, a small force of not more than four men should amply suffice to keep the two igarapés clean and to destroy the pools left by the falling waters.

A slight modification of the same measures would be necessary in the case of the Igarapé Tocos (Plan 2) and some of the small arms of the S. Vicente etc.

The ravine at the back of Rua Cearense (Plan 5) should be drained and the undergrowth and small trees destroyed. The washing-pools should be abolished altogether, and the inhabitants prohibited from using the waters for any purpose. Free drainage should be afforded by preventing the filling at the western end until the eastern section has been attended to.

THE DISEASES IN MANÁOS

The diseases which chiefly affect the death rate in Manáos are malaria, yellow fever, beri-beri, ankylostomiasis, dysentery and other infantile diarrhoeas, tuberculosis, bronchitis and pneumonia, typhoid fever and small-pox.

On none of the vast rivers flowing through the State of Amazonas are there any public hospitals, and the doctors practising in the settlements in the interior are few in number considering the vast extent of territory. Manáos has therefore to serve as a hospital base for all the up-river commerce.

Every steamer brings diseased persons from the interior of the State; many of them are already in the advanced stages of malaria, beri beri, ankylostomiasis, etc. Their health undermined by the ravages of malaria and the privations they have had to undergo, many of them suffering from lack of sufficient nourishment (for actual starvation is not an uncommon occurrence in the interior during the season of low river, when it is frequently impossible to send up provisions), these people often arrive in Manáos in a most precarious condition. Many are admitted to the wards of the Santa Casa de Misericordia in a moribund state and thereby raise the death-rate of the hospital and the city of Manáos The more fortunate ones make a very slow recovery, occupying the beds of the hospital for many days, and it speaks well for the medical and nursing staff that so many recover. We have seen as many as fifteen men from the Rio Madeira taken in an unconscious condition from a steamer, all suffering from pernicious malaria, almost pulseless, and yet with only a subsequent mortality of 60 per cent

MALARIA

An accurate estimate of the prevalence of malaria in Manaos from the cases in the hospitals is impossible for the reasons already given. If we confine our attention to those found suffering from the disease in the suburbs, we are again at fault, as many Amazonenses suffering as they do from malaria, on returning from the interior will go and live outside the city and so raise the number of persons found infected.

The Brazilian of the poorer class who happens to be a rubber collector is of a necessity a rover, and will frequently change his district, going up the different rivers as employment offers and acquiring fresh infections, 'fever' being considered a necessary evil as the result of having to work up-river.

If these people are not too ill they will go and live with their family or friends in a hut situated in an Anopheline region. Very little quinine is taken, though their blood may be laden with gametocytes. They lie about in hammocks with no mosquito nets, and consequently these 'gamete carriers' serve to infect *Cellia albimana* and *C. argyrotarsis*, which are the common Anophelines of the swamps in the outskirts of Manáos. Thus the districts can be infected with the more virulent parasites of malaria. Generally the huts occupied by these 'gamete carriers' are overcrowded with children and adults, and consequently at a later date the other inmates are attacked with the same form of fever. Our case books record many such instances. At Pará a very serious epidemic of malaria occurred among the very poor inhabitants of certain districts, in which Anophelines abounded, by the return of labourers who had come down from the Alcoboça and Madeira-Mamoré works, and who were suffering from very severe attacks of malignant tertian fever contracted in these districts. These labourers being heavily infected, and living under most abject conditions, served as efficient hosts for the parasites. Shortly after their arrival a great outbreak of malaria occurred, necessitating the appointment of a medical commission.*

If we compare the malarial localities of Manáos with the figures obtained from a mosquito survey, we find that all the heavily infected localities represent those areas in which the greatest number of Anophelines are present throughout the year. In the city proper the number of Anophelines is not so great as compared with those in the suburbs, and therefore the number of new cases of malaria is far less than in localities such as Mocò and Pensador. Moreover, the condition of the inhabitants of the city is better than that of the poorer natives living along the swamps.

During our residence in Manáos we have examined many children and adults living in the suburbs, and especially those in malarial localities. As far as possible spleen palpation and blood examination was practised, and latterly an examination of the faeces was always made. Children do not object to being palpated, whereas they view with apprehension the necessary preparations for making blood films.

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* Relatorio apresentado pala commissão nomeada para debellar a epidemia de impaludismo, reinante no Marco de Legua, Pedreira e Canudos. Belem. Imprensa official do Estado do Pará, 1909.

Moreover, a mother naturally objects to her infant's finger or ear being pricked. In order to obtain as many specimens as possible, free quinine, thymol, etc., was given to all indigent patients residing outside the city who were not attending the outdoor dispensary of the Santa Casa.

The following tables relate to a spleen and blood census practised on children from 6 months to 10 years of age.

APPARENTLY HEALTHY CHILDREN LIVING IN HUTS ALONG THE SWAMPS IN THE SUBURBS OF MANÁOS

Age	Children examined	Spleen palpable					Blood		
		I	II	III	Total	Percentage	Children examined	Parasites present	Percentage
6 months to 2 years	11	—	1	1	2	18·1	9	3	33·33
2 to 5 years ...	58	1	5	17	23	39·65	38	20	52·63
5 to 8 years ...	61	3	12	14	29	47·5	51	28	54·00
8 to 10 years ...	70	1	4	22	27	38·5	59	27	45·76

Total spleens examined...	200	Total bloods examined	157
Total spleens palpable	81	Total parasites present	78
Percentage palpable	45	Percentage infected ...	49·68

EXPLANATION.—I = Spleen greatly enlarged.
II = Spleen enlarged and edge projecting beyond ribs.
III = Spleen palpable but not projecting beyond ribs.

AILING CHILDREN—SAME LOCALITY

Age	Children examined	Spleen palpable					Blood		
		I	II	III	Total	Percentage	Children examined	Parasites present	Percentage
6 months to 2 years	45	3	4	11	18	40	43	20	46·51
2 to 5 years ...	81	4	15	22	41	50·62	72	39	54·16
5 to 8 years ...	68	4	11	20	35	51·47	38	23	60·53
8 to 10 years ...	76	6	12	13	31	40·78	25	13	52·00

Total spleens examined	270	Total bloods examined	178
Total spleens palpable	125	Total parasites present	95
Percentage palpable	46·29	Percentage infected ...	53·37

From these figures it is apparent that the swamps in the suburbs of Manáos are responsible for a considerable number of cases of malaria. Of a total number of 470 healthy and ailing children, the spleen was palpable in 206, or 43·82 per cent. Blood examination of 335 of the children showed malaria parasites to be present in 173, or 51·64 per cent. If we compare spleen and blood examinations made on children of the same ages, resident in Manáos and living near swampy and low-lying land such as Igarapés Bittencourt and Manáos, S. Vicente etc., the number of infected children is much the greater in the suburbs.

LIVING ALONG THE SWAMPS IN THE CITY

Age	Children examined	Spleen palpable					Blood		
		I	II	III	Total	Percentage	Children examined	Parasites present	Percentage
6 months to 2 years	21	1	—	1	2	9·5	18	5	27·77
2 to 5 years ...	64	1	3	5	9	14·06	47	14	29·78
5 to 8 years ...	33	3	7	4	14	42·4	33	18	54·55
8 to 10 years ...	59	1	6	12	19	32·20	49	22	44·89

Total spleens examined	177	Total bloods examined	147
Total palpable	44	Total parasites present	59
Percentage palpable	24·85	Percentage infected ...	40·13

COMPARISON OF MALARIA INDEX OF CHILDREN IN SUBURBS AND CITY

		Spleen rate		Blood rate	
		0–5 years	5–10 years	0–5 years	5–10 years
Swamps in Suburbs	Apparently healthy	36·23	42·74	48·93	50·00
	Ailing	46·82	45·83	51·80	57·14
	Average both classes	43·07	44·36	50·61	52·60
	City swamps ...	12·94	35·86	29·23	48·78

Thus we find that the spleen rate for the suburbs is 43·82 per cent. as compared with 24·85 per cent. for the city, and the malarial blood index in the suburbs is 51·64 per cent., in the city 40·13 per cent.

At five to eight years a greater percentage of children appear to show enlargement of the spleen and malarial parasites in their blood. Examination of 142 boys between the ages of ten and fifteen years, living in the suburbs, showed splenic enlargement in 51 cases, or 35·91 per cent.

Comparison of particular localities such as Moco, the Bosque, S. Raymundo, Colonia Oliveira Machado, where the natives are of the very poorest class, shows a much greater percentage of children with malarial infection. In these places, quite 70 to 90 per cent of the inmates of some of the huts are suffering from malaria. We had many opportunities of observing such conditions during the course of our work in the swamps, and many times have found whole families incapacitated.

On the other hand, our observations show that children and adults living in the city, and well removed from malarial swamps, remain free from malaria. Such non-malarial districts are not hard to find in Manáos, and as the sanitation of the city improves, so will the number and extent of these localities increase.

The majority of the Americans, English, French and Germans live in the business section of the city and remain free from malaria. When cases do occur, the infection can be quite easily traced. They have gone on a hunting or sailing expedition and exposed themselves to infection or are old '*imported cases*' from up river or other parts of the tropics. Many Brazilian clerks are employed by these firms, and it is noticeable how much 'fever' there is amongst those who reside near the outskirts of the city and adjacent to the swamps. A considerable percentage suffer from *sezões* (ague) at some time during the year.

During our residence in Manáos we remained free from malaria, and no quinine was taken unless we left the city to pursue our work in the swamps.

Many supposed cases of convulsions, dentition troubles, and tetanus in children occur during the year, and more especially in the months when malaria is particularly prevalent. From our observation of these cases we think they are largely of malarial origin.

As we show in the section on ankylostomiasis, 88 per cent. to 93 per cent. of the children living in the suburbs are infected with *Necator americanus*, and practically all our cases of malaria exhibited symptoms of this disease. It is not remarkable then that these children, their constitutions already weakened by ankylostomiasis, should be attacked and exhibit severe types of malaria.

The children who enter the Santa Casa are of course suffering from acute attacks of malaria, and exhibit the most severe symptoms of the disease. It is rare to find parents of the indigent class bringing their children to be treated for malaria; the gravity of the disease is not appreciated, and consequently the cases are well advanced. Blood examinations of the children admitted for various diseases show that 50 per cent. to 70 per cent. are infected with malarial parasites.

If a hut-to hut inspection in the swampy areas of the suburbs be made, from 20 per cent. to 80 per cent. of the adults will be found to suffer from malaria, the number varying according to the season of the year, condition of trade, and locality. Allowing for the *imported cases* living in these districts, we estimate that 20 per cent. to 55 per cent. of the population residing in Mocò, the Bosque, S. Raymundo, Colonia Oliveira Machado, part of the Igarapés Cachoeirinha, Castelhana, and western end of the Cearense Swamp suffer from malaria and can be ranked as *indigenous cases.*

These people may be attending the dispensary of the Santa Casa, but generally they do not do so, preferring to take an occasional dose of quinine or to treat themselves with one of the numerous proprietary medicines of which there is an enormous sale in North Brazil. It is quite common to find a poor native with four or five brands of pills in his possession, squandering the money which ought to provide him with nourishing food on a small phial of expensive 'silver-gilt' pills.

Malaria is most prevalent during the period of February to November; the number of cases begin to increase some two months after the commencement of the '*rains*' (p. 9) and the rise of the Rio Negro (vide chart). The mortality is greater during the months of June, July, and part of August. It is in these months that the wards of the Santa Casa are overcrowded and the cases of pernicious malaria most numerous. In this period many of the *imported cases* from up-river arrive in Manáos.

The parasites infecting the children and adults are *malignant tertian*, *benign tertian* and *quartan*, this last type being always imported. Malignant tertian (*Plasmodium praecox*) are the prevailing parasites, and their destructive effect can be witnessed on all sides;

in the children with chronic malaria, cerebral convulsions, etc. in the adults in the cachectic state and in the large number of pernicious cases. In children, crescents are not hard to find and this was the case with all our patients; quite 80 per cent. of our malignant tertian cases harboured gametocytes, often in very considerable numbers.

Benign tertian (*Plasmodium vivax*) is fairly common, and was found in about 20 per cent. to 25 per cent. of our patients. Many cases of double tertian infection, causing a quotidian type of fever, occur in Manáos, and a certain amount of confusion has occurred in the diagnoses until microscopic examination determined if the quotidian fever is due to *Pl. praecox* or *Pl. vivax.*

Quartan (*Plasmodium malariae*) parasites were rarely encountered in our patients; in fact, all our specimens were obtained from cases coming from the Rio Purus, Rio Japura, and Rio Madeira.

The clinical types of fever are quotidian, tertian, continuous and quartan. Bilious remittent fever type is not rare. Algid and cerebral types are quite common. Pneumonia, dysentery, haemorrhagic purpura, nephritis, neuritis, optic atrophy, have all been seen and are due to malignant tertian parasites.

Many of the rivers of the State are famed for the extreme malignancy of their malarial fevers and the resistance of these to quinine. The Rios Acre, Javary, Madeira and Coary have long been noted for such types of fever, and examples of their malignancy are to be met with in the Santa Casa Hospital. A quotidian type of fever, with an enormous number of crescents, resulting in an extreme degree of anaemia is encountered in cases coming from the Rio Madeira. Some of the doctors claim that there is a phenomenal resistance to quinine, but we cannot certify to this as quinine, as used in the Santa Casa, has worked wonders with these cases.

Blackwater fever. Cases of this disease are said to have been seen in Manáos. We have seen no case of genuine endemic haemoglobinuria in Manáos. In Iquitos and Manáos we have observed attacks in a man who had recently suffered in Africa from the disease. A special watch was kept for cases of this disease and the urines of all malignant malaria patients, especially those from the Javary and Madeira rivers were collected, but in none were we able to find any of the characteristic symptoms.

We desire to record the fact that since the last quarter of 1909 cases of typical blackwater fever have occurred amongst the people employed in constructing the Madeira-Mamoré Railway in the vicinity of Porto Velho on the Rio Madeira. During 1908 we had opportunity of observing many severe cases of malaria which were brought from the Madeira-Mamoré Railway to Manáos and treated in the wards of the Santa Casa, but we failed to find a case of blackwater amongst these men, either in the wards or post-mortem room. Among the working-force are Cubans and workmen, engineers, etc., brought from Cuba and the Isthmus of Panama, both blackwater fever regions. These men were brought to the Madeira river about 1908. It is of interest to record that the Booth Steamship Company* in its long connection with the Amazon region, never carried a case of blackwater until November, 1909, when a severe case was observed in a Spaniard who had been working up the Madeira river in the railway camp. We hear that other cases have been treated in Manáos (1909-10.)

YELLOW FEVER

This disease in 1856 attacked two-thirds of the population of the State; in 1861 another epidemic occurred. The fever appears to have been severe in 1899 and 1900.

For the foreigner this is the most serious of all the diseases to which he is liable in Manáos. The foreign population of the State is chiefly resident in Manáos, and the number of foreigners is sufficiently large to make the disease a very serious one.

We have already reported the overcrowding which prevails in certain of the streets of the city. These houses and barracks are mostly inhabited by Portuguese, Italian and Spanish labourers, and a great many of the newly arrived immigrants settle in these streets until employment is secured or a home arranged for.

We have shown that *Stegomyia calopus* abounds in all quarters of the city and that its existence in such numbers is due largely to the habits of the citizens. The foreigner is equally culpable, as his house or office provides numerous breeding places for the mosquito.

We acknowledge the efforts of the sanitary authorities to stamp out this disease. They inspect the houses and endeavour to abolish the breeding places of the Stegomyia. It is only when one attempts to fight this mosquito that a comprehension of the difficulties to be surmounted is really gained.

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* By permission of Dr. Melville Davidson, Medical Superintendent, Booth Steamship Company.

Stegomyia calopus is essentially a house mosquito. The female is not a 'rover' and keeps to a small area provided that she can obtain blood. She will lay her eggs in all kinds of receptacles capable of holding water, in fact, she often appears to choose the most unlikely places. The larvae and nymphae require a very small quantity of water and appear to be far more hardy than many of the other Culicinae.

The average dwelling-house or office in Manáos affords the Stegomyia an excellent propagating place receptacles containing water abound in the houses and backyards, underneath the floors, and in the various other places which we have already enumerated. The climatic conditions of Manáos are ideal for the breeding of the mosquito. There is plenty of rain, the humidity is considerable, the temperature is suitable, the breeding spots are ready. All of these conditions are present throughout the year. Certain months are especially favourable for the development of the young, viz.: September to January.

For the propagation of the disease there are numerous female Stegomyia all prepared to feed on a yellow fever patient. The opportunities for doing so are numerous, as the class mostly afflicted by this disease are careless in the use of the mosquito net If there is a net it may be torn or, more probably, it will not be used during the day. As has been so often verified, it is the young female *Stegomyia calopus* which, ravenous for food, flies about and bites during the day time. Hereafter she is more cautious and will bide her time, nature teaching her to bite during the gloom Hence, once the cycle period of twelve days is complete, she will be dangerous for any non-immune who lives in the city proper in an unscreened house or sleeps without a mosquito net.

It has already been proved that a certain temperature is requisite for the development of the cycle in the mosquito, and the maintenance of the virulence of the virus. The climate of Manáos favours this, as the preponderance of our cases of yellow fever occurred in or after the four hot months of the year.

Yellow fever can occur at all times of the year; usually the latter part of June, July and the first half of August are the months with few cases. Occasionally it has happened that we have seen no cases for four to six weeks, and consequently our experimental work has been seriously hampered From September to January the cases appear to increase gradually ; February and March may show a fall, and the number may continue to fall or rise again in the latter part of May.

The source from which the female Stegomyia obtains her infection is the labouring-class foreigner and the native child.

We deplore the custom of the laity in attempting to treat cases of fever occurring among their friends and relatives. The Portuguese, Italian and Spanish nationalities form the preponderating foreign labourer class in Manáos, and therefore they are the greatest offenders.

Should a foreigner, especially a new comer and non-immune, exhibit malaise, headache, pain in the back and lower limbs, a tendency to nausea and some fever, all early symptoms of the disease, instead of calling in a doctor to diagnose and treat the case, home remedies are given. Sometimes a purge is taken, but unfortunately it is not generally administered ; food is not withheld, and the patient may force himself to eat the most unsuitable food for one in his condition ; a sharp mustard plaster to the calf of each leg is applied and kept on until large blisters are formed. This is supposed to be an excellent remedy, and is almost universally used. The patient may lie in bed or attempt to go about his work ; in any case he is unscreened and exposed to the bites of the hungry Stegomyia thus during the first few days of his illness, the only period of the disease at which he is a source of infection, he serves to nourish and infect the numerous Stegomyia which have taken up their abode in his surroundings.

As the disease progresses and the symptoms become more and more defined, his condition becomes worse, and a doctor is called in at the end of the third or fourth day. Of what use is it to screen him ? The period of infection is passed, his blood is no longer infective, the appetite of the Stegomyia is already satiated. Probably the first three days of the disease are completed : the days which are so important for the doctor, for it is the management and treatment of a yellow fever patient during these three days which materially influence the ultimate result. Of what use is it to call a doctor or to come to the hospital if the patient is already in the throes of black vomit and exhibiting the prodromal symptoms of anuria? One of the saddest sights that can be continually seen in the hospitals is the entry of patients on the fourth to fifth day of the disease in a moribund condition. Many of these people would have recovered if only they had summoned a doctor or applied to the hospitals Numerous Stegomyia would have remained un infected and consequently there would be far less possibility of other foreigners and non-immunes becoming infected, as the number of infected Stegomyia would be greatly lessened.

Why should such a condition prevail? Is it the fault of the doctors and hospitals, the sanitary authorities, or the patient?

It is not the fault of the doctors and hospitals, as they continually urge the necessity for early treatment. It is not for lack of the necessary fees, because a doctor, after the first visit, will advise an indigent patient to go to the hospital. The hospitals are ready and willing. The Hospital da Benificencia Portugueza has a screened ward especially kept for the reception of yellow fever 'suspects,' where the patient remains until all doubts as to the diagnosis of the disease are cleared up.

The sanitary authorities publish from time to time the by-laws relating to yellow fever. All cases of the disease must be immediately reported under penalty of a fine. This is not sufficient The by laws should be amended to the effect that **all cases of fever occurring in non-immunes,** i.e., those persons who have not suffered from an attack of yellow fever, **should be notified within the first twenty-four hours.**

The screening* of all persons suffering from any form of fever should be made compulsory from the very onset of symptoms of fever.

All cases of fever in non-immunes must be examined by a doctor within twenty-four hours of onset of fever, and the diagnosis notified in writing to the Health authorities.

Foreigners unable to pay for a doctor, when suffering from fever, should on notification to the Department, be examined by the doctor appointed by the Sanitary Department, and, if found suffering from yellow fever, removed to the Hospitals.

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* We understand that there is an ordinance to this effect in the regulations of the Health Department of the City of New Orleans.

All foreign immigrants arriving in Manáos, should notify the Sanitary Department their place of residence, occupation or trade, and to each one should be delivered a copy of the by-laws relating to screening of fever cases.

The foreigners of the business class are accustomed to call in a doctor at once, as they realise the seriousness of yellow fever and the benefit derived from early treatment. The regulations would not inconvenience them, but they and their doctors would be compelled to send early notification of 'fever' cases, and the diagnosis made to the responsible authorities.

The classes that would benefit by the regulations would be the foreign labouring classes: the very ones who by their ignorance of the danger from the Stegomyia mosquito and the non screening of 'fever' cases, greatly assist in the continuance of yellow fever in Manáos.

We believe that after a few sharp fines had been administered a salutary lesson would have been learnt and the screening of all fever cases would be performed as a matter of course, even in houses where, under normal conditions, the use of mosquito nets was not universal. A further advantage would be that cases of malaria in the gametocyte stage would be protected from infecting the Anophelines.

In reality there would be very little hardship from such ordinance, as the majority of the Brazilian and foreign residents use mosquito nets. They are accustomed to use the net only during the night. Hence the law would compel the sick to be screened day and night. For the very poor residents who have not the money to afford a net the hospitals are the proper place.

We admit that the difficulties will be great, but only for a time. After a short period all the inhabitants, including the labouring-class foreigner, would become aware of the fact that non-compliance with the screening regulation entailed an immediate fine. Secondly, the non-immune would learn that the more the cases of fever remained unscreened, the greater would be the number of yellow fever cases, and for his own protection he would compel the 'fever' sick to use a net. Man is of a selfish nature, and the desire to live is predominant in every normal person. Let the lower classes learn to fear yellow fever, teach them the ways to avoid the disease, and one of the main difficulties will have been overcome.

Brazilians arriving from non yellow fever States occupy the same position as do the foreigners They are non immunes, and are susceptible to the disease. We have attended cases of the disease occurring in natives and Indians from the Acre and Purus districts, and in children and adults coming from southern parts of the Republic.

The Brazilian child is susceptible to yellow fever, and we have had opportunities of studying the disease in infants and young children from one to two years of age. The symptoms are of such a character that a diagnosis can only be made with extreme difficulty In the average case the mildness of the symptoms cannot attract the attention of the mother. Irritability, crying, mild bilious vomiting associated with slight fever in an infant only a few months old is almost always ascribed to gastric disturbances The more so as the infant recovers in a few days. With children there may be languor, possibly headache, disinclination to eat, mild fever, and possibly vomiting; the child may be up and moving about, its urine may or may not contain albumin and casts; thirst is always pronounced; vomiting may be absent, but it is only when 'black vomit' occurs that the parents have any idea of the child's disease.

With such vague and insidious symptoms it is not astonishing that no precautions are taken to prevent Stegomyia from biting these children.

We have been fortunate to see a number of mild cases resembling influenza, gastric disturbance, etc with fever of the types so well described by Marchoux and Simond * Our own attack in 1905 was very mild, resembling influenza and exhibiting no albumin in the urine, and yet we were protected and became an ordinary 'immune'

Many Brazilians suffer from most suspicious symptoms which, if occurring in a foreigner and non-immune, would be ascribed to an attack of yellow fever. It is worthy of note that these cases usually occur when there are other cases of yellow fever in houses in the neighbourhood. We hope to publish at a later date our observations of a series of these cases in adults and children and the infection resulting from them.

We do not think it necessary to present any clinical data regarding yellow fever, as the symptoms, etc., have been so often described It

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* Marchoux, E. et Simond, P. L.; Études sur la fièvre Jaune. Ann. de l'Inst Pasteur, Fev., 1906.

is our intention to defer the publication of our clinical report until we can present a more complete monograph embracing the pathological and experimental work of the laboratory. Autopsies have been practised on all the available material and careful records kept of the numerous cases seen during our residence in Manáos.

Ronald Ross[*] has always pointed out that 'a locality is malarious only when it contains persons already infected with the parasite, and also *sufficient numbers* of the proper species or varieties of Anophelines to carry infection to healthy persons.' Gorgas,[†] in his anti-yellow fever work, terms this the yellow-fever 'point,' and contends that a certain proportion of Stegomyia mosquitos must be present in a locality for the spread of yellow fever. If the number of Stegomyia remain above this 'point,' the locality will continue to have yellow fever as long as non-immunes are present, no matter how much fumigation is done or how carefully the sick are isolated. If anti-stegomyia measures are vigorously carried out and the Stegomyia so reduced in number that they are below this point, then yellow fever disappears Gorgas thus explains the success of the Havana and Panama measures, and the reason why so long a period elapsed between the active starting of the campaigns and the stamping out of yellow fever in these places.

Can Manáos be freed from yellow fever?

We see no reason why yellow fever should not be absolutely stamped out of the whole of the Amazon district and the cities of Pará and Manáos in Brazil and Iquitos in Peru freed of this scourge.

To attain freedom from yellow fever will take time and money, and it will require the active co-operation of the authorities, the Brazilian and the foreign residents of the city. Stegomyia will have to be continually attacked. War cannot be waged against this mosquito for a few months only. It must be fought throughout the whole year We do not have to go outside of Brazil to find an example of what can be accomplished in fighting yellow fever. The city of Rio de Janeiro was a veritable 'inferno,' but, thanks to Oswaldo Cruz, yellow fever is conquered. We know of no more striking example of what sanitation can accomplish than is evidenced by the mortality statistics of Rio. Only a few years ago thousands of deaths were due to yellow fever; to-day Rio de Janeiro is absolutely free of cases of this disease.

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[*] Ross, Ronald. Report on the prevention of malaria in Mauritius, 1909.

[†] Gorgas, W. C. Method of the spread of Yellow Fever. Proceedings of Canal Zone Medical Association, 1908.

The man who accomplished such a miraculous change experienced a difficult time. The citizens did not appreciate his methods and desires, his opponents were numerous and the methods they used were various. Only his dogged determination in continuing the anti-stegomyia measures saved Rio from remaining a plague spot. To-day his name is honoured, and the largest and most progressive Bacteriological and Medical Institute in South America is the Instituto de Oswaldo Cruz.

We do not believe that Stegomyia reduction will prove so difficult in Manáos as it was in Rio. It is not such an old city. The streets are wider, the buildings are not so cramped as in the old yellow fever quarter of Rio. Manáos has not the number of foreign emigrants that Rio had.

It is to the interest of the State of Amazonas that Manáos should be freed from yellow fever. The population of the State, considering the vast territory, is small. Immigrants of all nationalities are needed to develop the agricultural and cattle raising districts and to increase still further the commerce of the interior, and the rubber industry.

So long as there is even the suggestion of yellow fever occurring in Manáos the emigration to Amazonas will be greatly restricted. The peasant and artisan class of the Latin races will not care to emigrate to North Brazil as long as they know that the South of the Republic and the city of Rio means freedom from yellow fever. The State authorities must realise that every Portuguese or Spaniard falling ill, and perhaps dying of yellow fever, means a further blackening of the reputation of Manáos. Even if these people have no relatives in Manáos, their countrymen will record their deaths from this disease, and their relatives in Europe will magnify the dangers accordingly. The saying, 'give a dog a bad name and it will stick to him' applies in a special degree to Manáos.

Yellow fever is a preventible disease, and other nations look askance at those districts where yellow fever prevails. Year by year the number of these infected localities is decreasing and, as the numbers diminish, so will the demands for the eradicating of the remaining spots become a matter of international interest. It is certain that other yellow fever centres in Brazil will follow the example of Rio, Santos, etc. Let Manáos and Pará be in the front rank and prepared to reap the benefits which will occur from a yellow fever-free city and improved hygienic conditions.

Manáos possesses a Director and a Sanitary Service. They have done their best to improve the hygienic conditions and to stamp out yellow fever from the city. Much has been accomplished, and there has been a considerable diminution in the number of yellow fever cases. We have seen various foci abolished. Much work has been performed with little money.

If the present Director and *personelle* are allowed a fair amount of money and freedom of execution they will be able to put into operation the methods which have proved so successful in Rio, Havana, Panama and New Orleans.

Gorgas claims that the most expeditious and certain method of freeing a place from yellow fever is by waging war on the Stegomyia and reducing the numbers to below the yellow fever 'point.' Fumigation and isolation should be practised, but the main efforts should be concentrated on reducing the number of Stegomyia breeding in the place. In the long run this will prove less expensive and will be easier of execution in Manáos.

Manáos has a pipe-borne water supply and a good drainage system, two most valuable adjuncts in any anti-mosquito campaign. The area of Manáos proper is not extensive; the work can therefore be concentrated.

We have already experienced what can be accomplished by a small brigade. The Manáos Harbour, Limited, and the Booth Company in 1908 put one to three men on anti-mosquito work, specially to look after the precincts of their warehouses, offices, workshops, docks and lighters. We supervised the earlier inspections, and have verified the enormous diminution in the number of mosquitos and breeding spots. At first, the results were not satisfactory, as the mosquito 'point' had not been reached, but later on the figures were highly gratifying. If such measures were instituted on a larger scale, the benefits would be experienced by all the firms and residents of the neighbourhood.

ANKYLOSTOMIASIS

This is one of the most prevalent diseases in the State, and all parts of the Amazon region appear to be infected.

In 1906 we reported on the seriousness of the disease, as observed by us in Iquitos, Peru. We found all classes of the Peruvians to be infected, and we believed, from examination of the Indians, that the country right up to the base of the Andes was infected with this parasite.

In Manáos we have made a large number of examinations, and from our results we estimate that quite 88 per cent. of the population of the State of Amazonas harbour the parasite. All classes of the community show infection.

	Number examined	Number infected	Percentage
Hospital Patients—			
Children	193	180	93·26
Adults	783	691	88·25
Indigent Class—			
Children	452	433	95·79
Adults	398	394	98·99
Better Class—			
Children	419	405	96·65
Adults	490	378	77·14
Foreigners—			
Labouring class	187	123	65·77
Better class	69	28	40·57

Total examined	2,991	Children infected ...	95·67 %
Total infected	2,632	Adults infected	87·55 %
Percentage infected ...	87·99	Brazilians infected ...	90·71 %

These figures are in accord with the observations made in Iquitos, and show that the soil must be heavily infected.

The parasite has been found in infants of less than six months of age to old men of sixty-five years. The children in the suburbs appear to show severer infection and greater anaemia than children in the city. In the Santa Casa, typical examples of the disease are nearly always to be seen. The stunted, vacant-looking, bloated, anaemic child with a protuberant abdomen is a common sight. Severe anaemia and slight oedema are frequent symptoms in adults.

The general public do not appear to understand the dangers arising from infection with this nematode. All of our severe cases of malaria exhibited considerable intoxication due to this cause, which materially retarded convalescence. We have not to wait until symptoms are pronounced to realise how seriously the health of the people is influenced by harbouring ankylostomes. Both children and adults exhibit the lassitude, disinclination to work, the inability to continue strenuous exercise for long periods, the susceptibility to gastro-intestinal disturbances, the lowered resistance to disease and proneness to manifest severe, and sometimes grave, complications when attacked by mild diseases; all of which are the result of infection with *Necator americanus.*

This disease should be feared, because of the danger of neglect of treatment until the symptoms have become pronounced, or, more probably, because the constitution of the individual has been so weakened and undermined that he can fall an easy victim to any of the diseases prevailing in the State.

Malaria, tuberculosis, dysentery and other diseases are seen in particularly severe forms in patients evidencing the toxic symptoms caused by the worm.

The habits of the people are responsible for the spread and general infection of the soil by this parasite. Public latrines in Manáos are absent; and therefore vacant lots or side streets are used for the committing of nuisances. In the suburbs there exists no system of drainage; only a few of the houses possess dry earth closets or privies The natural functions must be performed; and therefore the ground in the neighbourhood of the habitations is soiled by the evacuations of the residents of the locality. The native usually chooses the belt of undergrowth at the edge of his clearing, but often in wet weather the earth immediately around or even inside the hut will be contaminated by the occupants.

The vicinity of the washing-pools, the undergrowth along the footpaths commonly used by the people, the banks of the igarapés and ravines are all contaminated by excreta.

The climatic conditions are favourable for the development of this worm, and the custom of the people to go about bare-footed or wearing light shoes causes the skin of the feet and ankles to be continually dirtied. The children during their earlier years run about naked, and are always rolling on the ground.

Some patients can give a history of ground itch occurring between the fingers and toes, but usually no such symptoms have been observed Children, especially of the poorer class, suffer from 'Amazon boils,' which occur about the ankles and lower part of the shins. They persist for some time and create large scars. It is possible that these boils are only an intensified form of ground itch which, through scratching and carelessness in cleansing, become infected with cocci and develop into chronic ulcers. We have examined portions of several ulcers, but nothing specific was observed.

We have been impressed with the severe symptoms present in many of our patients, in spite of the fact that they harboured comparatively few parasites, and we agree with other observers as to the toxic symptoms observable in these cases, which are altogether out of proportion to the number of worms found in the intestine.

Our patients have exhibited the ordinary symptoms noticed by all observers. We would call attention to a rather rare form. A patient in apparently good health will be seized with a rigor. The temperature will rise to 39·5° or 40° C., to fall after a few hours to normal or subnormal, or moderate fever may continue for one to two days. In one adult, two epileptiform seizures occurred, attended by a sharp rise of temperature. Examination of the blood will show a moderate amount of eosinophilia, but no evidences of malaria; there may be slight and transient albuminuria, and the faeces will contain an enormous number of ankylostomes. Thymol, an intestinal antiseptic, and small doses of calomel will quickly cure such patients.

Dirt eaters are not uncommon, and have been found at all ages. Children of six to eight years of age appear especially prone to acquire this habit. Other infected children may develop such idiosyncrasies as eating orange skins, chewing leather, or drinking filthy, even stinking, swamp water.

The mortality from this disease is uncertain, as many of the cases die from an intercurrent infection.

Ankylostomiasis is a satisfactory disease to treat, as efficient medication is generally followed by marked improvement in the condition of the patient, in which the outward and visible signs can be noticed by the patient and his friends. In the case of undersized, imbecile children, the improvement can be so marked that it must be seen to be believed. We have seen a veritable idiot transformed into a bright, active child: a change which impresses the patients, and is a valuable aid in persuading other patients to take treatment.

We have used thymol, beta-naphthol and Mons' mixture of chloroform-eucalyptus. For children we prefer to give thymol or beta-naphthol in small doses, as the drowsy condition induced by the chloroform-eucalyptus mixture is apt to alarm the parents. When there is much weakness, oedema, or cardiac dilatation present, the treatment must be carried out with caution. Beta-naphthol has not acted well in our hands and is not to be compared with thymol, which is an excellent and reliable drug. The simple precautions which are advised for thymol medication should be observed; we have never seen ill effects, and the patients are rapidly cleared of ankylostomes. Only severe cases require to be treated in the hospitals. We have found it advisable to superintend the treatment of a person in a particular district, and when he is on the way to recovery to appoint him to supervise the treatment of the other local patients.

This is a disease which should be actively combated by the State. Treatment on a large scale should be carried out on the lines of the Porto Rican campaign. Malaria and ankylostomiasis should be fought together, as one disease influences the other. We have the advantage that both are suited for treatment in clinics, where the patients cannot have much medical supervision. Severe types of the disease should on no account be treated in the district clinics: they are the suitable cases for admittance into the hospitals.

BERI-BERI

In the hospitals a great number of cases of this disease are admitted from the river steamers arriving from the interior of the State. The Acre, Purus and Javary districts furnish many cases. Manáos proper, as well as the suburbs, supplies many examples of this malady.

The oedematous type is the most common, a mixed oedematous and paralytic form, and a pure atrophic type, are both frequently seen. The galloping type occurs, and was of considerable interest to us in the year 1905, and again in 1907, because of its almost epidemic character. In these years we noted more cases of beri-beri coming from the interior of the State and suburbs of Manaos. It has seemed to us that beri-beri is increasing in the city, the suburbs, and the interior of the State.

We have seen no cases in children, though they are said to occur, all our patients having been from eighteen to forty years old. Over 90 per cent. were males. Brazilians are the chief sufferers from this disease, but Portuguese and Spaniards of the labouring class are also liable to become attacked.

The better-class foreigner rarely acquires beri-beri. No doubt this is largely due to the housing conditions and their preference for ordinary European diet.

The Brazilians, so long as they avoid overcrowding, and live in the city under hygienic conditions, and partake of a liberal and varied diet, remain free from the disease. When the better class foreigner or Brazilian exposes himself by living in overcrowded quarters, amid unhygienic surroundings, on a poor and monotonous diet, his physical condition lowered by attacks of malaria, ankylostomiasis or dysentery, the possibilities of his acquiring beri-beri are very great.

We do not intend to discuss the etiology of this disease. The following points, however, may be of interest:—

Beri-beri is frequent in places where rice forms an insignificant portion of the diet.* In the gaol, where 25 per cent. of the deaths are due to the disease, the prisoners only eat beans and dried goat's meat imported from Rio Grande do Sul and Uruguay. Very little rice is used in the Santa Casa. The poorer class, in the suburbs and in the interior of the State, prefer farinha (mandioca meal) to rice. Beans, salt-cod, dried meat, plantains, farinha and fruit constitute the diet of many families, and yet beri-beri occurs. Much of the material used up-river is old and mouldy, but this does not apply to Manáos.

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*Reply received from Dr. Hamilton Rice, F.R.G.S.: Beri-beri met with in Indians.

1. District?

Uaupes river, tributary Rio Negro, N.W. Amazons valley, Colombia and Brazil.

2. Villages on river or inland?

On the river or upon igarapés leading into main stream.

3. Tribe of Indians?

Guainanos, Tupi Stock.

4. If natives come in contact with white people, and if so have these whites brought beri-beri into the district?

With a few caucheros who are inhabitants of the district, whose intercourse with other whites is extremely restricted, nor is there evidence of their ever having brought beri-beri into the country.

5. Nature of food of natives?

Fish, monkeys, agoutis, tapirs, farinha, yuca, pupunhas, plantains and caxiri, a beer brewed from mandioca, large quantities of ahi, a very strong red pepper, are used.

Husked rice is used in Amazonas and is imported in sacks from Rio de Janeiro (arrôs national) and from Europe (arrôs carolino). It is well washed before being boiled, and is cooked for a long time.

We have devoted considerable time to the investigation of this disease, but with few encouraging results. A large series of blood cultures; a series of cultures of the patient's own colon bacilli, for agglutination with his blood serum, and cultures of organs at early post-mortem, and attempts to infect chimpanzees with rice or with food soiled with the faeces of beri-beri cases, constitute the principal

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6. Do they take rice?
   No.
7. If no rice, what local farinaceous food?
   Principally farinha and yuca.
8. Do they obtain salt meat or fish? Can salt be obtained locally?
   Fish dried in the sun and preserved in tough, fibrous leaves for varying periods of time is used extensively. Salt obtained from traders and possibly from Indians of the Kerary, though doubtful.
9. Roughly give diet of natives?
   Consume very large quantities of caxiri. Sun-dried fish eaten raw, and fish broths seasoned to a fiery strength with ahi, into which are dipped pieces of baked cassava bread. Green boiled plantains and farinha uncooked or boiled to a viscous consistency known as mingai, and baked or stewed pupunhas. This varied from time to time with tapir, monkey, agouti, paca, peccary, and worms.
10. Number of cases seen?
    Three. Two men, 35 and 45 years old; one woman, 20 years old.
11. Native ideas of the disease? (Do the natives desert the sick and move to another village or hut?).
    Apparently unable to differentiate one disease from another even though different symptoms manifest themselves. Sick not deserted.
12. Is there a possibility of beri-beri having been introduced from some infected district.
    No evidence of such.
13. Forms of Beri-beri?
    Paralytic. Galloping.
14. Treatment by natives?
    Removed to the darkest, least used or unoccupied portion of the maloca to be cared for by the wife or nearest women relatives. Hammock slung very low to ground. Pages or medicine men practice their ritual of passes, incantations, sucking and spitting to exorcise the Evil Spirit, but eventually desist as the patient grows worse and the poor wretch is left to fare as best he can.

part of our work. No definite conclusions can at present be deduced from the data acquired.

More fruitful results were obtained from the treatment of beri-beri patients. The removal to a clean dwelling, disinfection of furniture and clothes, a diet consisting largely of milk, raw eggs and raw beef juice, small doses of calomel followed by intestinal antiseptics salol or beta-naphthol—expulsion of ankylostomes with thymol, and massage, have benefited patients who could not afford to leave the State through pressure of business.

The universal practice in Manáos is to send the patient away immediately the diagnosis of beri-beri has been established. The Brazilians are sent to the Southern States of the Republic, the foreigner to Europe. Delay of a week or two very often ends in a funeral instead of a sea voyage. Poor patients are assisted by the State or sent to Itacoatiara, a small place a few hours from Manáos Patients with chronic debilitating diseases are advised not to remain in the wards of the Santa Casa, where they expose themselves to contact with cases of beri-beri.

The etiology of beri-beri is so obscure that it is highly desirable to collect as many data as possible regarding the disease in Brazil. The Société de Pathologie exotique, Paris, has appointed a Commission to enquire and collect information on the subject. North Brazil is one of the beri-beri centres, and is interested in the problem of the etiology of the disease. Much valuable information could be furnished by the States of Amazonas and Pará. We would recommend the appointment of a Commission in Manáos to collect data relating to cases of beri-beri occurring in the city and suburbs.

DYSENTERY

Amoebic dysentery is not exceedingly common in Manáos, and the majority of cases appear to occur in the suburbs, especially along the Igarapé de Cachoeirinha. The disease usually runs a severe course

Bacillary dysenteries are numerous in Manáos, and are particularly prevalent during the months of February to August. Most of the cases come from the suburbs, where there is no drainage system, and where the soil is polluted by the evacuations of the inhabitants. The infection is generally of a mixed bacillary form, but we have had patients suffering from a pure Shiga or Flexner type, agglutinating the specific sera. Vaillard's anti-dysenteric serum was used with most gratifying results in several acute cases. Unfortunately we were only able to import a small supply.

ABSCESS OF LIVER

Amoebic dysentery furnishes a few cases annually. We have to record the extremely rare complication of liver abscess following a severe attack of yellow fever. Two cases occurred, and from one a bacillus of the para-colon type was isolated from the pus. Both patients had exhibited exceptionally severe icterus, which had persisted for some months after recovery from the original disease. The abscesses were noted six and ten weeks respectively after convalescence. No history of dysentery was obtainable, nor was it probable that these patients had suffered from the disease.

DIARRHOEAS OF CHILDREN

Infantile diarrhoeas are of frequent occurrence in Manáos, and are the cause of a high mortality. The suburban child is probably already infected with malaria and ankylostomes, and therefore falls an easy victim to any gastro-intestinal affection. The diet of the average child of the respectable poor is ill-adapted to sustain and nourish its bodily requirements.

We have examined the faeces of many children suffering from severe diarrhoeas, and have found, in a child of four years of age, enormous numbers of Tyroglyphines, which were voided with every evacuation. Specimens were sent to England for identification, but the tubes were mislaid, and when discovered were so penetrated by mould that identification was impossible. We have had two cases in adults suffering from acute enteritis, accompanied by the discharge of much blood; in the blood clots hundreds of small acarines were moving about. Both patients remembered that they had eaten a large amount of fruit, over-ripe bananas, etc., and one of them had subsisted on mouldy farinha. All the patients were rid of these pests by irrigations of boric acid and eucalyptus. We have seen acarines in the faeces of people who had partaken of much cheese. These were probably *Tyroglyphus siro* L., and in these latter cases no inconvenience was experienced from the presence of the parasites Railliet, in his Traité de Zoologie, p. 691, states that acarines have been found by various observers in the stools of children suffering from dysentery. We believe these acarines are responsible for some of the severe diarrhoeas seen in children and the point would be worth investigating. One must remember, however, that varieties of these tiny mites abound on wood and other things, and caution is advisable before deciding that Tyroglyphus can produce diarrhoeas In the case of the child and one of the men, we adopted every precaution and found the parasites in successive stools and the returned fluids of the clysters.

TYPHOID FEVER

In 1907 we performed an autopsy on a Brazilian who had died from typhoid fever. A small outbreak of the disease occurred in 1908. Cases of para typhoid were seen in 1908; the blood of the patients reacted typically to cultures of para-typhoid A. We hope at a later date to publish observations on a number of typhoid, para typhoid and para-colon infections which occurred in Manáos during 1907 and 1908.

Bacteriological examinations were made from time to time of the water-supply of the city, the cheap ice-creams and fruit drinks. The results are to be embodied in a later report to the authorities

TUBERCULOSIS

Pulmonary tuberculosis is unfortunately a disease which appears to be gaining a strong foothold in Manáos and the poorer class shows a steadily increasing percentage of infection.

Overcrowding is a serious evil in Manáos This can hardly be avoided as the population has increased far more rapidly than has the house accommodation.

Rents are very high, and consequently many families have to live in small houses where overcrowding must occur. Many of the houses are badly lighted and ventilated, and possess dark inner rooms in which several people sleep.

Promiscuous expectoration is a habit which is practised by the majority of the people as can be verified in public places and in cafés. In the hospital great difficulty is experienced in controlling the public patients from spitting about the wards.

The authorities have a separate barrack for indigent patients suffering from tuberculosis. Unfortunately before this building was inaugurated some of the sisters of the order of Santa Anna became infected through nursing in the wards of the Santa Casa.

Tuberculosis appears to run a rapid course among the poorer class natives as is to be expected since they suffer from overcrowding, insufficient and inappropriate food. Dried goat's meat, salt cod and beans constitute a poor diet for patients suffering from this disease.

We believe from the clinical and pathological material at our disposal that tuberculosis is already proving one of those dangerous diseases which are attacking the people of the interior of the State.

Many of our cases were found to be suffering from malaria, and practically all of the indigent class harboured *Necator americanus.* With a combination of three such depressing diseases the prognosis for such patients is very gloomy.

A league against tuberculosis is active in Rio de Janeiro and it is to be hoped that branches will be formed in the cities of Northern Brazil, so that the people may learn the elements of hygiene.

PULMONARY DISEASES

Bronchitis and Pneumonia are frequently met with in the rainy season. The prognosis is grave in cases of pneumonia.

Pneumonia occurring in patients suffering from malaria is rare; the cases seen were all infected with malignant tertian parasites. The mortality is high. Pneumonia as a complication of yellow fever is fortunately rare. Lobar pneumonia occurred in nine of our cases, and was responsible for seven deaths.

WHOOPING COUGH

Epidemics of this complaint attack the children in Manáos and in the interior. In Iquitos, Peru, in 1906 we found over 50 per cent. of the children had the disease, and that it was causing serious loss of life amongst the Indians in Peru. Broncho-pneumonia is a serious complication and causes a high mortality.

STOMATITIS

We have seen several cases amongst children. All were complicated with septic pneumonia which proved fatal. We have seen malignant stomatitis in a man from the interior. The disease involved the cheeks, gums, tongue and probably the posterior nares

We have seen no cases of measles, diphtheria or scarlet fever.

LEPROSY

A moderate number of cases occur among the residents of Manáos Early cases have been seen where infection is said to have taken place in the Purus district. We have seen both forms of the disease, many anaesthetic ulceration cases were examined and the presence of the bacilli verified.

A small lazaretto would be a comfort for the lepers as some of them are in a wretched state.

SMALLPOX

The State furnishes free vaccine for a large number of the inhabitants. The sanitary department is to be congratulated on the success of their preventive and quarantine measures which have effectually checked the spreading of this disease on the various occasions when it has been introduced from the South of Brazil, Pará, or from up-river places.

ELEPHANTIASIS

We have seen several cases in which the foot was involved. The majority of the patients come from the southern parts of Brazil or Barbadoes.

CHYLURIA

Two cases of this disease were seen, both of which had been acquired in other countries.

GOUNDOU

One case of old Goundou was seen in a Brazilian. The man had been in Africa.

RECOMMENDATIONS

We suggest that the sanitary authorities be empowered:—

I. (*a*) To make a spleen census of all children in the city and suburbs of Manáos, and that this should be repeated every three to six months.

(*b*) That at the same time the haemoglobin percentage of all children should be ascertained, and those showing marked anaemia should have their faeces examined for ankylostomes.

(*c*) That all children suffering from enlarged spleens or ankylostomes should be given free quinine and thymol.

(*d*) That a supply of quinine tablets, specially coloured in order to prevent the sale of the State quinine, should be given to each school teacher or responsible person in the localities of the poor, and that such quinine should be available for the inhabitants of the locality.

(*e*) That, once a week, a free clinic should be held in each of the large districts of S. Raymundo, Colonia Oliveira Machado, Mocò and Pensador or Flores. That so far as possible attention should be directed to the treatment of malaria and cases of ankylostomiasis. It should be remembered that what the department desires to attain, is to cure the children and adults of malaria and rid them of ankylostomes. *Simple preparations of quinine, thymol, arsenic and iron are quite as efficient as expensive combinations of the same.*

Severe cases of these diseases and patients suffering from other ailments should be directed to apply for treatment to the outdoor department or wards of the Santa Casa de Misericordia.

II. (*a*) That separate cards should be published by the State giving a few simple facts about malaria, ankylostomiasis and tuberculosis and emphasising the dangers incurred by neglecting treatment.

(*b*) That one of these cards appropriate for the disease from which he is suffering should be filled in with the name of the patient and should be given him to use as his identification card for the suburban clinic and hospital dispensary.

(*c*) That every school should have posters on the wall depicting and giving facts about malaria and Anophelines, yellow fever and Stegomyia, ankylostomiasis, and tuberculosis.

(*d*) It should be made compulsory for the teachers of every class to teach the pupils the meaning of the posters, and simple facts in hygiene, and these facts should be revised by all the scholars at least once a year.

III. That a special and permanent force of men should be formed to act as a mosquito brigade.

(*a*) To destroy *Stegomyia calopus* breeding-places in the city proper.

(*b*) To inspect and report on the condition of every house and office within the city proper, noting the general cleanliness of the place and the amount of rubbish.

(*c*) That all rubbish should be removed free of charge after the first inspection, but that later collections of rubbish should be removed at the expense of the householder.

(*d*) That all yards and houses should be inspected monthly, and that extra inspections should be made of houses that are apt to be neglected and dirty. If found in such a condition the tenants should be summarily fined. That special inspections should be made of barracks inhabited by many people.

(*e*) That each householder should be compelled to affix in a prominent place the ordinances promulgated by the authorities concerning screening of cisterns and tubs, breeding of mosquitos, collections of rubbish and the screening and notification of all patients suffering from fever.

(*f*) That all places where building is going on should be inspected, and the builders fined if found guilty of breaking the by-laws

IV.—MALARIA WORK.

(*a*) The Igarapés Manáos and Bittencourt should be attended to during falling and low river as already advised, p. 22.

(*b*) Avenida 13 de Maio (Plan 6 and 25) the swamps to be kept oiled until filled and drained.

(*c*) Marsh in Ravine Cearense (Plan 5) to be kept oiled until filled and drained.

(*d*) The hollows on certain streets should be filled.

V. (*a*) That the men already attached to the various public squares, should be held responsible for not allowing water to collect in the fountains.

(*b*) That the men already appointed to look after the roads and gutters within the city proper, should be held responsible for not allowing stagnant water to collect in the gutters.

We believe* that a permanent force of 30 men would suffice to carry out the anti-stegomyia measures and to inspect the places within the city where Anopheles are continually breeding. This force would include:—

1 Head Inspector.
3 Inspectors or *Moustiquiers.*†
1 Head Foreman.
5 Foremen.
17 Labourers.
1 Storeman.
1 Driver.
1 Clerk.

This would allow for 3 gangs each of 1 foreman and 3 labourers.
2 „ „ 1 „ „ 4 „

For some months in the year, the entire force would be able to devote their whole attention to combating Stegomyia, as the igarapés would require very little treatment during rising river. As the sanitary authorities have had a force of 13 men at work the additional expense will not be so great. We suggest that only energetic men who will take an interest in their work should be appointed as inspectors and foremen.

We suggest that the prisoners should be employed in removing the grass, weeds, pools, etc., from the swamps in the vicinity of the gaol.

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* For the various details in organising such a force, the measures recommended by Major Ross for fighting malaria in the Island of Mauritius can be modified to suit the local conditions.

† Ross, Ronald, *loc. cit.*, p. 113.

EXPLANATION OF PLAN OF MANAOS

1. Igarapé Sao Vicente. Formerly Stegomyia bred here plentifully in a large and deep pool, with many *Cellia albimana* and *Ce. argyrotarsis*, *Culex fatigans*, *Mansonia titillans* and *Uranotaenia geometrica*.

2. Igarapé behind Rua Luiz Antony. Fair number of Anophelines

3. Plano inclinado. Many Anophelines.

4. Igarapé behind Rua Monsenhor Coutinho. Many Anophelines, some malaria.

5. Swamp behind Rua Cearense. Badly infected with malaria. Anophelines abundant.

6. Swamp along Avenida 13 de Maio. As in No. 1.

7. Portuguese Hospital. In neighbourhood of Nos 6 and 8

8. Swamp and igarapé (creek) along Rua Dr. Machado. Many Anophelines, some malaria.

9. Upper end of Igarapé Bittencourt. Many Anophelines, much malaria.

10. Large swamps, many Anophelines and much malaria.

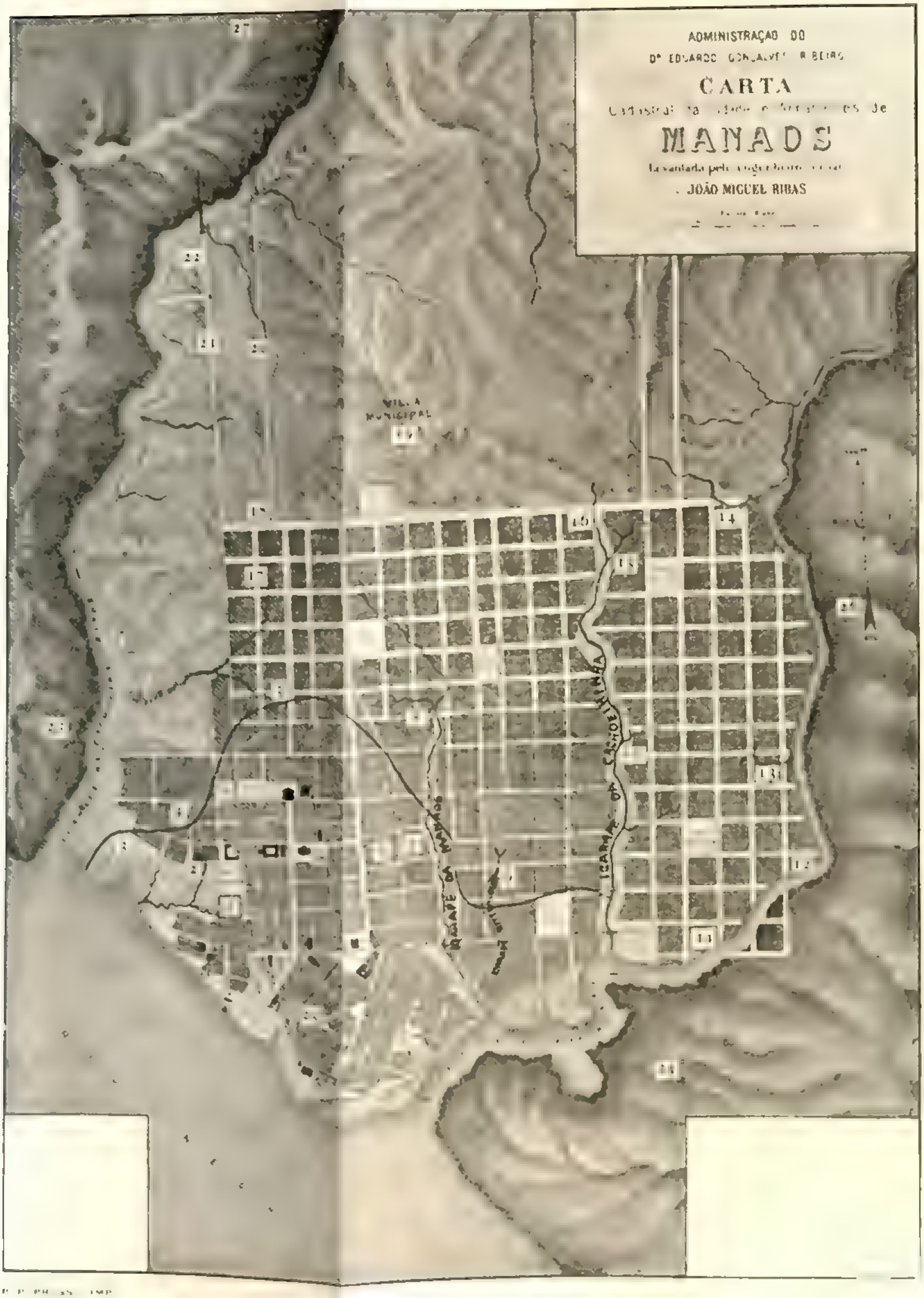
11 to 15. Large swamps. Many persisting throughout the year Very heavily infected with malaria; abundant Anophelines and *Mansonia titillans*. Many of the poor inhabitants reside along these swamps.

16. Same conditions, but not so marked as Nos. 11 to 15.

17. Igarapé Castelhano. Heavily infected with malaria; Anophelines abundant. Many ***Uranotaenia pulcherrima*** captured.

18. Swamps. Not quite so heavily infected with malaria as No. 17.

19. Villa Municipal. Swamps abundant in neighbourhood and many cases of malaria in adjacent houses.


ADMINISTRAÇÃO DO
Dr EDUARDO GONÇALVES RIBEIRO
CARTA
Cadastral da cidade e arrabaldes de
MANAOS
levantada pelo engenheiro civil
JOÃO MIGUEL RIBAS
VILLA MUNICIPAL
IGARAPÉ DA CACHOEIRINHA
IGARAPÉ DE MANAOS
27
22
21
17
14
13
12
11
23
24
25

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20 to 22. Swamps very extensive. Many Anophelines and numerous cases of malaria. Near the wheel house of the waterworks several examples of *Megarhinus separatus* and *Mansonia longipalpis* were captured.

23. São Raymundo. District contains many swamps and is heavily infected with malaria. *Cellia albimana* and *C. argyrotarsus* very abundant. Many of the very poor inhabitants reside in the district.

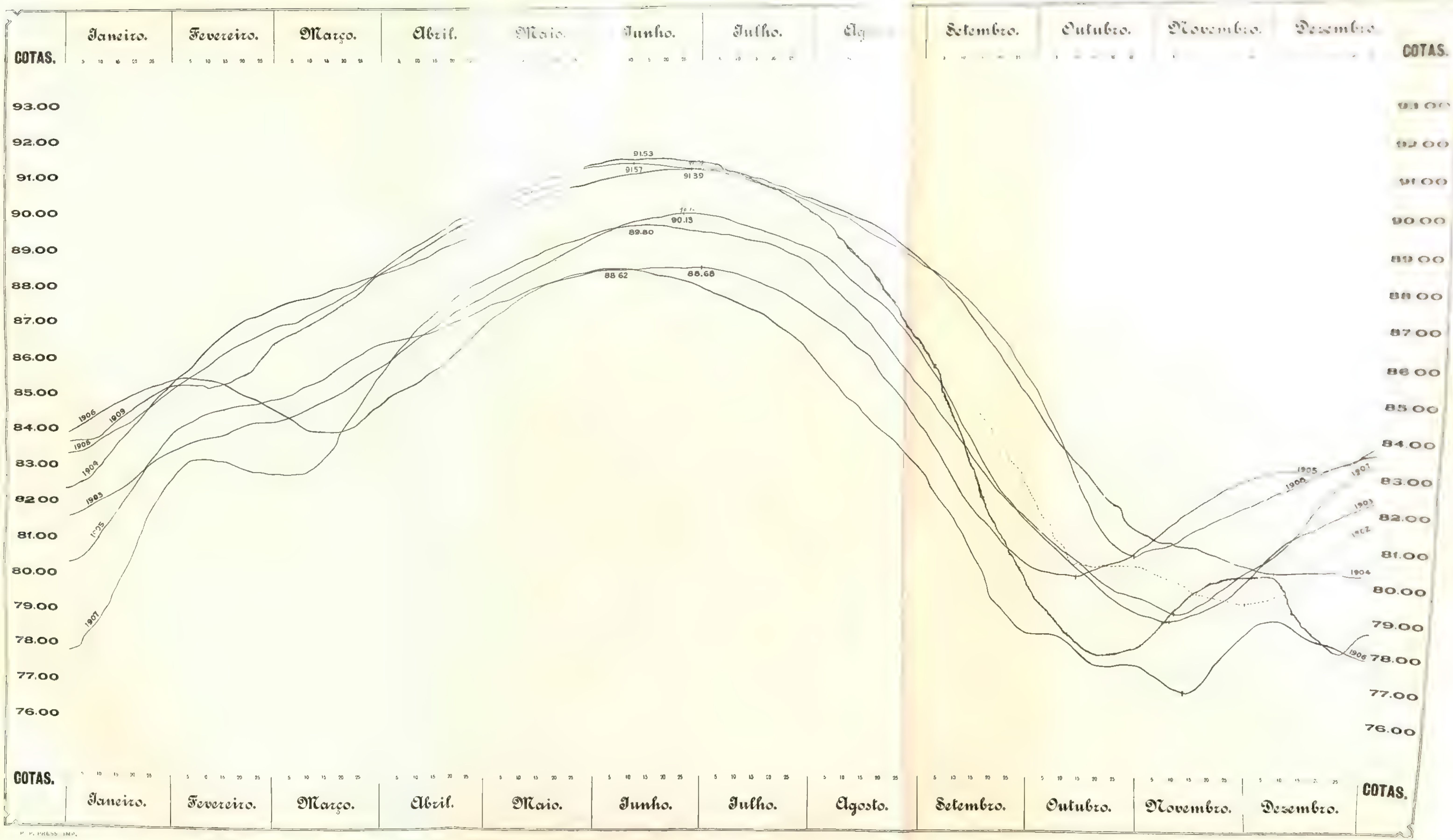
24. Colonia Oliveira Machado. Same conditions and class of residents as in No. 23.

25. Low land near Rua dos Andrades. Many *Stegomyia calopus*, *Mansonia titillans* and *Culex fatigans*. Many cases of yellow fever have occurred in the neighbourhood. A great number of newly arrived Portuguese, Italians and Spaniards reside in the houses, and create a serious condition of affairs through the overcrowding of the houses and their unhygenic habits, and the presence also of a number of unscreened water barrels.

26. Swamps. Heavily infected with malaria.

27. The Bosque, Pensador and Flores swamps are extensive. Many *Uranotaenia lowii* and *Ur. geometrica*. The Anophelines are very plentiful, and many severe cases of malaria occur amongst the residents living near these swamps.

The curved line traced near Numbers 3, 4, 5, 7, 9, to the Igarapé da Cachoeirinha roughly outlines the yellow fever area. Between this outline and the river front is the most densely populated section of Manáos, and the majority of the non-immune population reside within these limits. Included in this area is the older portion of the city. Outside of the traced outline many more Brazilians reside, and the blocks of streets are not so densely populated.



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Manáos Harbour, Ltd.

Graphico do Fluxo e Refluxo do Rio Negro.

THE PATHOLOGICAL REPORT OF A CASE OF ŒSOPHAGOSTOMIASIS IN MAN

BY

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AND THE

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Expedition to the Amazon, 1905-1909.

(Plates I, II, III, IV, V, V^A, V^B)

Œsophagostomiasis is a disease produced by a small nematode, an œsophagostome, of which there are several species. The larvae of this worm become encysted in the coats of the bowel, usually in the large intestine, and gradually develop into immature adult œsophagostomes. They can rupture their cyst walls and pass into the lumen of the intestine or into the peritoneal cavity.

Anthropoid apes, certain species of monkeys, and some of the domestic animals can be infected by various œsophagostomes. In a monkey it is not at all uncommon to find small subperitoneal nodules and submucous cysts containing minute worms. Weinberg[*] has given a very complete report of the macroscopical and microscopical appearances of the disease in anthropoid apes and monkeys Œsophagostomiasis is an extremely rare disease in man. Brumpt reported the first case in 1905, in a negro from the Omo river near Lake Rudolph in East Africa.

Railliet and Henry described the œsophagostome in this case, naming it *Oes. brumpti.* Our case is the second to be recorded in man, and from the conditions found at the autopsy there can be no doubt that the patient died from a septic peritonitis due to the lesions caused by this worm.

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[*] Weinberg, M. Œsophagostomose des anthropoïdes et des singes inférieurs. Archives de parasitologie, T. XIII, No. 2, 1909.

The worms taken from the cysts were sent to Professor Railliet,[*] who most kindly volunteered to undertake the description of the parasite.

The history of the disease is unknown, as the patient entered the public ward of the Santa Casa de Misericordia in Manáos in an extremely critical condition. He was admitted to the Hospital suffering from acute dysentery; he became delirious, and died within three days. Our records describe him as a man, aet. circ 36, a native of the Amazon State, Rio Purus region.

The autopsy was performed ten hours after death. Unfortunately the case occurred at a time when there were many other duties to be performed, and consequently the post-mortem was incomplete.

Body of an extremely emaciated man; colour mulatto; muscles well developed; scars on both tibiae from old boils. Subcutaneous fat very pale and scanty. Muscle fibres pale, brownish red and dry.

Thorax. Lungs emphysematous, slight hypostatic congestion of left. Pericardium contains two spoonfuls of cloudy flocculent fluid. Heart: left side slightly enlarged, muscle shows some hypertrophy, pale and friable. Several small recent atheromatous patches along aortic ring; mitral valves competent, very slight thickening; semilunar valves normal. No other atheromatous patches seen.

Abdomen. On opening the peritoneal cavity, the omentum and some coils of small intestine were found matted together on the right side, attached to the caecum and to the ascending colon by tough adhesions. The small intestine was distended with gas. On separating some adhesions, tiny pockets of pus opened. Surface of ileum, caecum and ascending colon dotted with small nodules. Spleen not enlarged, marked perisplenitis, capsule of organ on anterior superior surface had a large pinkish white area the size of a florin, cartilaginous, 2 to 3 mm. in thickness; no adhesions between it and diaphragm.

Liver slightly enlarged; few adhesions between surface and diaphragm; capsule dull, not thickened; organ pale, friable, cloudy swelling, commencing to decompose.

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[*] Railliet, A. et Henry, A.; Étude zoologique de l'œsophagostome de Thomas. Annals of Tropical Medicine and Parasitology, p. 89, Vol. IV, No. 1, 1910.

Kidneys slightly enlarged, very soft, especially the right; cloudy swelling. Decomposition advanced.

As the adhesions were very firm, the small intestine, caecum, ascending and transverse colon, were taken out *en masse* and placed in Kaiserling's fluid for later examination.

The following description of the affected bowel was made some months after it had been in Kaiserling's fluid:—

SMALL INTESTINE

Exterior.

The surface of the small intestine, extending from the ileo-caecal valve, for a length of about one metre, is studded with small raised, dark coloured tumours; the majority of these involve the lower part of the ileum for a distance of 35 cm. from the ileo-caecal valve. The nodules are less numerous and pronounced in the remaining 65 cm. of the infected bowel.

Thirty-seven well-marked tumours can be counted. The greater number of them appear to be lying between the external muscular layer and the peritoneal covering of the bowel. The surfaces of the nodules are usually smooth, but some few are roughened and covered with plastic adhesions. The majority of these growths are small and oval in shape, and vary from the size of a small pin's head to 7 mm. by 9 mm. and elevated as much as 6 mm. to 8 mm. above the surface of the bowel (Plate IV, fig. 2). Some are flatter and more button-shaped (Plate V, fig. 5). Others again are elongated, leech-like masses. These latter (eight in number) measure from 14 mm. to 23 mm. in length and 6 mm. to 11 mm. in width; they project 4 mm. to 9 mm. above the surface; their base is somewhat constricted so that the contents of the cyst cause a bulging of the tumour walls (Plate IV, fig. 1).

The nodules are opaque and greyish-black in colour, with sometimes a few light ochre-coloured points in or underneath the cyst wall.

The situation of the nodules varies. Some lie along the mesenteric attachment; others are on the sides or upper surface of the gut. The larger tumours appear to lie on the upper wall and parallel to the longitudinal axis of the bowel; some few, chiefly situated along the mesenteric attachment, are placed transversely.

The nodules are found either 4 cm. to 12 cm. apart or in small groups of three to five oval tumours separated by 1·5 to 2·5 cm from one another. Occasionally, small clusters of two or three are seen lying close together (Plate V, figs. 3, 5, 6).

Many of the smaller tumours are hard and shot like; some few are calcareous. The larger ones are soft, compressible and doughy, pitting freely on pressure. The tumours appear to be firmly attached to the wall of the intestinal tube. Provided the walls of the cyst are intact, very great pressure is needed to cause a rupture.

In three of the tumours, the outline of a coiled worm can be seen lying underneath the tightly stretched membranous covering.

At one place along the upper part of the ileum there is a triangular prolongation of the external muscular coat and peritoneum, and, from the apex, the ends of two small worms can be seen projecting. (Plate IV, fig. 2; fig. 4 shows the same × 8). On examining the attachment of the mesentery to the ileum, a worm was found penetrating the mesentery; it had escaped from its cyst in the external muscular layer and still had its cephalic end within the cyst cavity (Plate V, fig 10, × 8).

On opening these nodules a small worm is found lying in a semi-solid dark brown mixture. Each cyst contains one worm; in no instance have two worms been found in one cyst. One tumour may be composed of two and, in some cases, three to four cysts. The worm is found lying either coiled and twisted or stretched out

Very often the head is damaged in pulling out the worm, as if its cephalic end had been embedded and fixed in the surrounding tissue. The cyst wall appears to consist of the peritoneal coat, and the floor, of fibres of the external muscular layer (p. 68). A small cyst which has been opened—the two ends of the worm being partially drawn out—is seen in Plate IV, fig. 2, and the same × 8 in Plate IV, fig. 3.

The intestinal tube was distended with gas, and at two places the bowel appeared to be sacculated, with a constriction at the lower ends of the pouches (p. 61). The walls seemed greatly thinned and very transparent, and, by transmitted light, other nodules could be seen attached to the inside of the bowel.

The ileum at its junction with the caecum is very contorted, and surrounded by a mass of fibrous adhesions, omentum, appendices epiploicae, etc. In separating the ileum, a cyst, the size of a large pea, was ruptured, and an immature adult œsophagostome escaped. In the mass of adhesions, two other small cysts were found, but these were free from any connection with either caecum, colon or ileum.

Interior.

On opening the small intestine, some twenty nodules are seen situated in the walls, the majority of them causing a distinct bulging of the mucous membrane.

The greater number of the tumours are small and oval in shape, measuring 4 mm. by 6 mm., up to 5 mm. by 9 mm. Some few have an elongated form, 15 mm. by 6 mm. (Plate IV, fig. 2). Though causing this bulging of the mucous membrane, none of the tumours is so markedly elevated as those nodules situated on the external surface of the intestine. The average bulging of the mucosa, produced by the cysts, is from 1 mm. to 6 mm.

A few of the nodules on the external surface are seen to cause a bulging of the mucous membrane into the lumen of the bowel. They are all more or less flat tumours with broad bases (Plate V, fig. 5). (The opened cyst with a worm partially drawn out is one with these characters, Plate IV, fig. 2.) The long leech-like tumours (Plate IV, fig. 1) produce no bulgings into the lumen of the canal; they are nearly all situated over the areas of Peyer's patches, which are enlarged and slightly inflamed.

The tumours in the submucosa lie transversely or diagonally, a few longitudinally to the axis of the bowel, and the majority of them are situated along the attachment of the mesentery. These adhere firmly to the wall, and are generally harder than those on the external surface.

The walls of the bowel are unusually thin in places. At 23 cm. and 47 cm. from the ileo-caecal valve are two pouched areas (p. 60); the lumen of the bowel is considerably enlarged, and beyond the sacculations, are constrictions of the canal. Examination shows that the constrictions cause an actual narrowing of the lumen of the gut, forming partial strictures, so that it is difficult to introduce the tip of the little finger. Dilatation of the tube in the proximity of the constrictions has resulted in the formation of the two pouches. The narrowing is due in the one case (pouch 23 cm. from ileo caecal valve to a large cystic tumour lying parallel to the longitudinal axis of the bowel and attached to that portion of the wall furthest away from the attachment of the mesentery. In the other case (pouch 47 cm from ileo-caecal valve) two medium sized cysts, which have apparently coalesced, are lying diagonally across the wall, and directly opposite them is another cyst lying lengthwise. In both cases the cysts have burrowed or fastened on to the two side walls, drawing and puckering them together and resulting in the constriction of the lumen. A few medium sized cysts are attached to the walls of the dilated areas. It is probable that these pouches have resulted from the weakening of the walls by the damage caused by the cystic tumours and the over-distension of the canal: the intestinal contents, being hindered by the obstruction, tend to collect, and, through the peristaltic pressure from behind, dilatation of the already weakened and inelastic wall ensues.

CAECUM

Exterior (Plate I, Plate II, fig. 1).

The walls of the caecum are mottled with bluish-black, slightly raised, irregularly shaped areas, especially along the anterior and posterior longitudinal muscular bands. These areas are so closely approximated as to appear in places as one wide mass; closer inspection shows them to be a series of subperitoneal cysts covered over with thickened peritoneum.

Radiating from the longitudinal bands are transverse striae (Plate I), 4 mm. to 9 mm. broad, which stand out prominently by reason of their darker colouring. Along these areas are dull ochre-coloured, irregularly-shaped spots, varying in size from a pinpoint to 4 mm. to 7 mm.; some of these are very prominent. Other striae are outlined by a series of small ochre-coloured nodules the size of small shot, and are elevated 1 mm. to 3 mm. above the surface.

Three dark oval elevated tumours, measuring 6 mm. by 11 mm to 13 mm. in length, and from 3 mm. to 5 mm. in height, are attached near the posterior band. They are similar to the ones on the outer surface of the ileum.

The walls of the caecum are very thick and rigid especially about the anterior band. On holding the opened up caecum to the light the dark areas can be seen as thickened masses, involving the greater part of the walls (Plate II, figs. 1 and 2).

Incision of the dark or ochre-coloured nodules shows various sized cavities containing immature adult œsophagostomes; the dark contents appear to be simply degenerated blood cells. The ochre-coloured cysts contain degenerated erythrocytes, and pus cells with many bacteria; the spots in the walls of the cysts are patches of necrosis or calcareous deposits (Plate II, fig. 1). Here, again, each cyst contains only one worm. The majority of these cysts appear to have originated in the subperitoneal fascia and to have spread into the external muscular layer. The peritoneal covering is enormously thickened by very old and tough adhesions which are difficult to strip off without rupturing some cysts.

Appendix vermiformis (Plate II, figs. 1 and 2).

The appendix at its junction with the caecum is surrounded on two sides, by a firm, dark-bluish tumour mass which so distorts its orifice as to make it almost impossible to pass a probe through the lumen into the caecal cavity. On opening up the canal the tumour mass is found to consist of a series of five cystic subperitoneal tumours, involving the wall of the caecum, and of a couple of large submucosal cysts which have invaded the neighbouring plica sigmoidea, with consequent bulging of the mucosa. The wall of the caecum at the junction is thickened and honeycombed with the five small subperitoneal cysts, each containing a worm.

About 1 cm. from the tip of the appendix is a bean-shaped nodule, 8 mm. by 10 mm., which is growing from the subperitoneal fascia of the wall. It does not involve the lumen. Microscopic examination reveals two small cysts, which are situated near the large one but are developed in the submucosa.

ASCENDING COLON

Exterior.

The walls of the ascending colon for its whole length are covered with thickened adhesions containing enlarged glands, fat and omental tissue. On attempting to strip the walls of these masses, many small, dark-coloured, cystic tumours are ruptured and small worms disclosed. So numerous are the tumours that it is impossible to remove the adhesions completely without spoiling the specimen.

The same dark-coloured, slightly raised areas are present, but are not so extensive as those developing in the walls of the caecum The anterior longitudinal band is almost free from these areas and nodules. The posterior band is thickened and appears to be infiltrated along the whole of its length by hard masses. On the internal and posterior walls are eighteen small oval cystic tumours.

At two places between the internal and posterior bands the appendices epiploicae are thickened, and dissection exposes a small worm lying on the wall of the colon. In each case the worm is seen lying near the ruptured cyst from which it has evidently recently issued. The worm is lying on the wall of the bowel, free from its restraining cyst, and apparently only confined by the adhesions of the epiploica to the wall. The fascia around the worm is stained dark-brown, and microscopic examination shows very many red blood cells, some pus cells, and numerous cocci, streptococci and short colon-like bacilli. [Plate V, fig. 7, appendix epiploica turned back and worm in situ. The three light points seen near the worm are portions of worms belonging to other cysts. The covering over these cysts is very thin, and was torn when turning back the epiploica. The exit-hole made by the œsophagostome is seen as a dark spot above and slightly to the right; specimen × 16.]

Caecum (Plate II, fig. 2; Plate III, fig. 1).

Interior.

On examining the interior of the caecum, a number of cord-like, ribbed, opaque masses attract the attention. They infiltrate the walls of the gut, usually following and involving the plicae sigmoideae, and, by their development in the submucosa, cause the mucous membrane to project prominently into the caecal cavity. These cord-like masses radiate from one muscular band to another, the anterior and posterior bands being particularly involved. As depicted in Plate III, fig. 2, these rows of cystic tumours, while well separated from one another, tend to merge into a common centre and form compact masses, especially about the anterior and posterior bands; the internal band is less affected. In between these radiating rows of cysts, other cyst tumours are present, so that there is very little free wall-area unaffected. The coalescing of so many tumours produces at these centres a great thickening of the wall, as is well seen in Plate II, fig. 2, and Plate III, fig. 1.

The cysts composing these strands are of varying dimensions, measuring 8 mm. by 3 mm. to 40 mm. by 4 to 7 mm. It is difficult to determine the exact length of some of the tumours without opening them. Some of the largest tumours, on being incised, are found to consist of a row of five to seven equal-sized cysts. Others again, with irregular outlines, and all the appearances of a series of cysts lying end-on-end, prove to be composed of one or two long cysts.

The cysts are soft and very compressible, differing from the harder cysts encountered in the ileum. Many of the spongy cysts have their walls dotted with yellowish areas. A few of these areas are actual sloughs, and quite moderate pressure suffices to rupture the necrosed wall. There are no calcareous patches. The bulging of the mucosa caused by these cysts is pronounced and varies from 3 to 5 mm. up to 9 mm. along the plica sigmoideae. Plate III, fig. 1 depicts a medium sized distended cyst, and through the thinned and tightly stretched mucosal covering the outlines of a large worm can be easily seen. This cyst was continuous with a large cyst in the external muscular coat, which bulged inwards as well as outwards.

On making a cross-section through the caecum, a striking picture is obtained. The wall in the vicinity of the longitudinal bands is thickened by dense fibrous tissue and honeycombed by a number of cysts of different sizes. Many of these cysts have been cut through, and parts of worms are lying in the cyst cavities (Plate V, fig. 2). The cysts appear to be situated in the subperitoneal and submucosal fascia; some lie between the internal and external muscular coats of the bowel. Some cross-sections show that all three forms of cysts have developed through being superimposed on each other. In such a case, the submucosal and intramuscular cysts appear to be of older and the subperitoneal cysts of more recent growth. The cysts with much cicatrical tissue around them are usually empty and may be partially calcified.

The thickness of the wall varies. At the anterior band it is 6 mm. to 9 mm., and in betwen the clusters of cysts the bowel wall is thinned and very transparent (Plate II, fig. 2).

Ileo-caecal valve.

The segments of the valve are thickened and infiltrated by submucosal cysts. The edges of the upper segment are distorted by five, the lower segment by two small cysts. The bases of the segments and floor of the gut are infiltrated by nine medium cysts. The plica sigmoidea, opposite the valve, is invaded by a large cyst in the submucosa.

Colon. (Interior.) Plate I; Plate III, fig. 2.

Of the 32 cm. of the ascending colon invaded by these tumours, the first 13 cm. exhibit the greatest changes. Tumours of all shapes and sizes are occupying the walls and floor of the gut, where, developing in the submucosa, they have caused prominent bulgings of the mucous membrane. Some of the tumours are the size of small nuts, measuring 17 mm. by 8 mm. and 9 mm. thick. Many of the tumours are oval in shape, others have the character of the leech-like cysts seen on the outside of the ileum (cf. Plate IV, figs. 1 and 2). Other tumours are bunched together or arranged in long rows, infiltrating and distorting the plicae sigmoideae.

Raised, yellowish patches are seen in some of the cyst walls; the mucous membrane surrounding them is ulcerated. Examination shows these necrotic areas to be small abscesses. The long tumours are soft and fluctuating, and present the usual characters of the submucous cysts.

The cyst tumours are developed on the sides and floor of the bowel, the majority being arranged transversely to the axis of the colon; the posterior wall appears to be the most infiltrated. In between the areas of cystic tumours the tissues of the walls appear thinned and inelastic.

A cross-section of the colon 14 cm. from the ileo-caecal valve, and through an infiltrated part, shows the wall to be thickened from 6 mm. to 11 mm. and to present the same honeycombed appearance observed in the caecal walls (Plate I; Plate V, fig. 1). The same subperitoneal, intramuscular and submucosal cysts are seen; the cysts appear to be closer together. In a cross-section 24 mm. long, seven cysts (five subperitoneal, two submucosal) were exposed; each cavity contained parts of a worm. No calcareous deposits were found, but microscopic examination showed several foci. If the bowel wall at the side of a submucous cyst be incised, little difficulty is experienced in shelling out the cyst intact, which then appears as a thin membranous sac containing a worm. It is impossible to shell out old submucous cysts with much fibrous tissue, or subperitoneal cysts, without rupture of the wall.

MICROSCOPICAL EXAMINATION

Nodules from various parts of the small and large intestine were imbedded in celloidin or paraffin and, where possible, serial sections were made. Many of the nodules in the wall of the caecum and colon required decalcification before imbedding. The serial paraffin sections were affixed to the slide with glucose-dextrose solution and, after drying, were passed through toluol and alcohol and flooded with thin celloidin. The film was allowed to harden and the slide was then placed in water to dissolve the glucose; the sections protected by the thin celloidin covering floated off and could be manipulated with ease. Van Gieson and Weigert's elastic tissue stains were found of service.

ILEUM

Oval submucosal cyst. Cut transversely.

The cyst has developed in the submucosa; though distended the cyst does not appear to press on either the muscularis mucosae or the internal muscular coat. The walls of the cyst are of thickened connective tissue. The cystic contents appear to consist almost entirely of degenerated erythrocytes; a few large mononuclears are present. In some of the sections portions of a worm are seen cut across. The cyst wall is infiltrated with numerous mononucleated cells and some small round lymphoid cells.

Examination with a higher power, shows that the cystic contents contain a few perfectly preserved red blood cells, and a few large mononucleated polygonal-shaped cells which contain a few pigment granules. Small clumps of granular pigment are scattered about. The cross-section of the worm shows that there are many degenerated as well as some normal red cells in the intestine. The cyst wall is infiltrated by large mononuclears and granulation cells, the infiltration being more dense in the inner part of the walls. The side abutting on the internal muscular coat is especially infiltrated, the infiltration extending halfway through the internal muscular coat. The tissue of the submucosa exhibits a slight infiltration of mononuclear leucocytes, but the muscularis mucosae and mucosa are entirely free. The vessels in the cyst area are engorged with blood; many of them contain numerous mononuclear leucocytes. Traversing the internal and external muscular coats are large cells containing pigment granules; none of these cells is noticed in the mucosa. No eosinophiles are to be found.

Oval submucous cyst with necrotic patch on wall projecting prominently into intestinal canal.

The cyst has developed in the submucosa and has involved the inner fibres of the internal muscular coat. The distension is extreme and has caused a very prominent bulging of the mucous membrane. The fibres of the muscularis mucosae are not atrophied but appear to be thinned. The necrotic patch in the mucous membrane proves to be only a superficial ulceration which does not extend to the deeper layers nor involve the muscularis mucosae. There is very little infiltration of the internal muscular coat or of the tissues in the immediate vicinity of the cyst. The contents of the cyst comprise large mononuclears, some polymorphonuclears, a few large hyaline cells and some pigment-containing cells, these latter are situated at the periphery of the cyst. The red blood cells are nearly all degenerated A longitudinal section of a worm is present and in the intestine several polymorphonuclears, mononuclears and a few degenerated red blood cells are seen. The walls of the cyst have a well-marked necrotic lining, in which are a few polymorphonuclear leucocytes. The connective tissue of walls is infiltrated by large mononucleated cells, and a considerable number of eosinophiles. Pigmented cells are seen in rows, directed towards the subperitoneum. The intermuscular space contains very distended blood vessels.

Subperitoneal cyst; leech-like tumour; longitudinal section.

Serial sections show that the cyst has developed in the loose areolar tissue of the subperitoneal space and that the fibres of the external muscular coat are not involved nor atrophied. The tumour has everted the peritoneum and has so developed that the sides and ends bulge and overhang like a mushroom. The cavity of the cyst measures over 8 mm. in length. The walls of the tumour are formed of thickened connective tissue which is greatly hypertrophied at the two ends. The cyst is not partitioned and the cavity is filled from end to end with many degenerated cells, some red cells, mononuclear leucocytes, and two sections of a worm, a transverse and a longitudinal one. The thickened cyst walls, especially the two end and basal walls, are infiltrated with leucocytes.

Near one end of the cyst, is a well-defined necrotic area which occupies the whole thickness of the internal muscular coat; the submucosa and internal fibres of the external muscular coat in the vicinity of this necrosis are infiltrated by large mononuclear cells.

Lying in the cavity are masses staining lightly with eosin and possessing no nuclei. Some are long elongated bands which appear to consist of degenerated fibres of some kind. With high magnification, they resemble the cuticular remains from the moult of the worm. The red cells are grouped around the worm, but none is seen inside it. The mononuclears and large polygonal cells are most numerous at the sides of the cavity. The tissues of the retaining walls immediately around the cyst are infiltrated by rows of large mononuclears and small lymphocytes; eosinophiles are very numerous; only a few large pigmented cells are found. Less infiltration and fewer eosinophiles are noted in the outer part of the walls The end walls of the cyst show extensive thickening with very little infiltration in the outer layers, but, further in, the tissues are densely infiltrated. The external muscular coat is infiltrated by a few mononuclears which extend to the intermuscular space.

The circumscribed necrotic area in the internal muscular coat is filled with many large mononuclears, a few erythrocytes and large hyaline cells containing a little pigment. Outside the necrotic area, is an intense infiltration of small round cells and large mononucleated cells which extend through the entire coat and involve the intermuscular space and the contiguous fibres of the external muscular coat; on the other side they penetrate the submucosa, where the infiltration is not so great, and does not extend to the muscularis mucosae.

In another leech-like cyst the development has begun in the outer fibres of the external muscular coat and extended to the subperitoneal space. The cyst is more distended and the cells bulge prominently. The parietal wall is covered with layers of plastic exudate. The cystic contents have more red blood cells and a considerable number of pigmented cells and free pigment granules: very few eosinophiles are seen. The infiltration of the walls is less marked and more small round cells are present. No masses resembling the cuticular remains of the moult of the parasite are to be found. A small haemorrhagic cyst lies adjacent, in the submucosa, distending the space and lifting up the muscularis mucosae; the fibres of the internal muscular coat adjoining the cyst are somewhat atrophied, and there is considerable infiltration of this layer with mononuclears, large pigmented cells, a few eosinophiles and large giant cells. The contents of the cyst are composed of masses of degenerated and some few normal red blood cells and pigmented leucocytes. A cross section of a worm shows the intestine to be crowded with more or less well preserved erythrocytes and some pigment granules. The pigmented leucocytes appear in little rows extending towards and into the subperitoneal space. The mucous membrane, though distended, is intact. The iron reaction is easily obtained.

Section of two small nodules, lying close together on the surface of the ileum.

These cysts are entirely separated from one another. They have originated in the external muscular coat and have distended the subperitoneum. The larger one has invaded the whole thickness of the external and extends to the outer half of the internal muscular coat; the smaller one is limited to the outer fibres of the external muscular coat, and does not greatly distend the subperitoneum. The larger cyst contains the usual cells, the pigment granules are quite numerous, but few pigmented cells are seen. Two sections of a worm occur. The infiltration of the cyst wall is not extensive, and very few eosinophiles are present. The small cyst contains numerous polymorphonuclears and a few pigmented leucocytes; the erythrocytes are very hard to find. No section of a worm is seen. The cyst wall of the external side is necrotic and infiltrated with polymorphonuclears and small round cells; on the internal or visceral side, the wall is densely infiltrated with large clumps of eosinophiles, some small lymphocytes, and a few pigment-containing cells.

Cyst in the internal and external muscular coats.

This cyst creates a bulging both on the inside and outside of the bowel. It is dumb-bell-shaped and involves the inner half of the external coat and the whole of the internal muscular coat; it is situated diagonally to the longitudinal axis of the bowel. The two broad bases are joined by a narrower part or funnel. A section of a worm is lying in the internal part. The contents consist of many pigmented leucocytes and a large number of erythrocytes and pigment granules. In the cavity of the cyst, in the internal muscular portion several very large giant cells containing pigment granules are present. The cyst walls, composed of muscular fibres, are extensively infiltrated by mononucleated leucocytes and granulation cells; the part in the internal coat contains several giant cells. The lymphatics to the subperitoneum contain many mononuclear pigmented leucocytes. The vessels in the intermuscular space are filled with mononuclear leucocytes and normal looking red blood cells. Some of the veins are compressed by the extensive infiltration.

Cyst in the external muscular coat over a Peyer's patch.

The cyst extends through the whole of the external and part of the internal muscular coat; the infiltration is extensive and reaches as far as the submucosal space. The contents of the cyst are leucocytic, and do not present any unusual characters; no giant cells are seen. The walls are infiltrated by large mononuclears, some small lymphocytes, and a very few eosinophiles; the infiltration extends to the middle of the submucosa, but no cells have passed the muscularis mucosae. The epithelial cells of the mucous membrane are swollen, and many are slightly necrotic; the lymphoid tissue of the glands is congested and small mononucleated leucocytes are quite numerous.

Other portions of the ileum which, apparently, contain a submucosal cyst, on microscopical examination, are found to consist of collections of small tumours which lie closely together, but are absolutely distinct. It is not unusual to find two or three small cysts in the submucosa and one, rarely two, in the internal muscular coat. It is quite common to find submucosal and external muscular cysts in the same piece of bowel, and these, by their invasion of other coats, closely approximate one another. On only one occasion have there been found cysts in each of the coats, viz., submucous, internal and external muscular coats. In this respect the cysts of the large intestine differ, as it is most common to find cysts situated in the different coats lying close to one another.

On examining sections from three different portions of the ileum, in addition to the ordinary cysts containing large oesophagostome larvae, minute larvae of a nematode are found situated in various parts of the bowel wall. In one piece many larvae are situated in the subperitoneal space, cut transversely, or a portion of one is lying stretched out, or again, a coil or two are to be seen. They occur in the neighbourhood of the blood vessels, but none is seen within. In the second piece of gut, the large cyst invades the whole of the internal muscular coat, part of the external muscular coat and a portion of the submucosal space. Directly under the muscularis mucosae but outside the cyst are seven portions of minute larvae in cross-section. In the third portion little sections of minute larvae are present in the external muscular coat and in some of the sections numerous cross-sections of larvae are found in the deeper part of the submucosa. Weinberg* describes minute larvae in the muscular coats of the large intestine, but they appear to be surrounded by a fibrous sheath which is not seen in any of the sections which I have examined. I submitted some sections to Dr. Weinberg,† but he was unable to give an opinion regarding the cross-sections of these small larvae.

CAECUM

Cord-like tumour of caecal wall.

This proves to be a very elongated submucosal cyst, occupying the entire space of the submucosa, and involving the inner half of the internal muscular coat, and an intermuscular cyst invading both muscular coats, and infiltrating but not distending the subperitoneal space. The fibres of the muscularis mucosae are somewhat atrophied and the mucous membrane displaced.

Large mononuclears, polymorphonuclears and enormous vesicular cells, and a few eosinophiles with products of degeneration form the cell contents of the cyst. erythrocytes can only be found with great difficulty. Two transverse sections of the worm show many polymorphonuclear and a few mononuclear leucocytes in the interior of the intestine. The cyst walls are extensively infiltrated with mononuclear and lymphoid cells; thick rows of eosinophiles occupy the middle layers and extend with the infiltration of mononuclears and small normal cells to the muscularis mucosae. The internal muscular coat and intermuscular space show the same degree of infiltration. In the deeper part of the submucosa, in the immediate neighbourhood of the cyst, are two large giant cells occupying the centre of a clump of small round cells. No pigmentation of the leucocytes is observed. Small clumps of minute bacilli are present in the cyst cavity.

The cyst in the internal and external muscular coats resembles an hour-glass, the constricting area being at the level of the intermuscular space. The contents are rich in degenerated erythrocytes and a few pigmented mononuclears. A large pigmented giant cell is situated alongside a longitudinal section of the head of a worm. The walls are infiltrated almost exclusively with small round cells and some mononuclears. The vessels in the neighbourhood are filled with erythrocytes and mononucleated leucocytes. The infiltration in the subperitoneal space is not intense and consists of mononuclears and a few small round cells. Minute traces of pigment are observed in many of the leucocytes collected about the small veins; no pigmented cells are seen inside the vessels.

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* Weinberg, M. *loc. cit.*, p. 193.

† 'Il n'est pas douteux que les grosses larves sont celles de l'œsophagostome. Elles resemblent beaucoup à ce qui je trouve dans mes preparations.'

'A côté d'une grosse larve se trouve, comme vous l'avez vu, un œuf. Seulement, il est tellement imprégné de matière colorante qu'il est impossible de voir sa structure. Il ne faudra cependant pas vous étonner si un jour vous trouvez dans un nodule hémorrhagique un nématode complètement adulte. C'est exceptionnel, mais cela existe. Vous trouverez l'indication d'un cas semblable au bas d'une page de mon travail d'ensemble (p. 202) Archives de Parasitologie.'

'Il est intéressant que dans la paroi de vos nodules il existe une forte infiltration par des cellules éosinophiles, ce qui n'existe pas toujours chez les singes.'

'Je n'ai jamis vu de nodules parasitaires dans les ganglions lymphatiques. Je les ai vus dans la paroi de l'estomac, dans la paroi abdominal, dans le diaphragme.'

'Il est impossible de dire si les petites larves, dont on voit un très grand nombre dans la partie profonde de la sous-muqueuse, sont bien les larves d'Oesophagostome; c'est possible mais je ne puis pas l'affirmer.' (April, 1910.)

Cord-like tumour with small necrotic patch in mucous membrane.

It was found impossible to cut out the mass from the wall of the caecum without dividing a cyst in the subperitoneum. A small immature female œsophagostome was drawn out. Section of wall was 24 mm. long, the tumours occupied 20 mm. The submucosal tumour consists of a series of cysts occurring in a group of three small submucosal cysts lying alongside one another, a fourth and larger cyst occupying the entire submucosal space, a fifth cyst in the internal and external muscular coats, a sixth and minute one in the submucosa, the seventh being a slightly larger one in the submucosa and involving some fibres of the internal muscular coat. On the peritoneal side of the section is seen the subperitoneal cyst divided, and near at hand, a dark brown mass occurring in the loose tissues of the subperitoneum.

The contents of the group of three tumours vary considerably. The outer cyst is composed almost entirely of mononuclear leucocytes and a few pigment cells. A diagonal section of a worm contains a few mononuclears. The outer half of the adjoining walls are infiltrated with eosinophiles. The second cyst contains a number of partially degenerated erythrocytes and some large mononuclear and pigmented leucocytes, the transverse section of the worm showing that there are many erythrocytes in the intestine, and slight pigmentation of some of the cells lining the gut; the walls are infiltrated with many more mononuclear leucocytes than the first cyst, and contain many eosinophiles; pigmented mononuclears are seen traversing the muscular coat.

The two cysts lie very close together and do not distend the submucosal space.

The third cyst contains no worm, and its contents consist chiefly of polymorphonuclear leucocytes and a few large pigmented mononuclears, and, in the wall next to the fourth or larger cyst, are several large peripherally pigmented giant cells. The tissues of this wall are necrotic, especially the outer ones, a condition due to the extensive necrotic condition of the fourth cyst. The infiltration is particularly intense about the wall adjacent to the internal muscular coat.

The fourth cyst contains two cross sections of a worm and exhibits all the characters of an abscess. Pus cells are numerous; many Gram-positive cocci and short bacilli are present in the cyst and also in the intestine of the worm. The walls are necrotic and the necrosis extends to the mucosa, where there is a small necrotic ulcer. The infiltration of mononuclear and polynuclear leucocytes is very extensive, and part of it extends to the fifth cyst, which is situated in the two adjoining halves of the internal and external muscular coats. The number of eosinophiles present is scanty, and no pigment or erythrocytes can be seen.

The fifth cyst occupies the outer half of the internal and the inner half of the external muscular coat. The cavity is small, and is filled with mononuclears and a few large polygonal non-pigmented nucleated cells. A cross-section of a worm contains only mononuclear cells. The muscular walls of the cyst are moderately infiltrated with mononuclear, small round cells and a few eosinophiles.

The sixth cyst is a minute one in the middle of the submucosa. It is free on all sides, and does not exhibit any great infiltration of the cyst walls. Red blood cells, many of them well preserved, a scanty number of mononuclears, free pigment granules, and a few pigmented leucocytes comprise the contents of this cyst. A cross-section of a small worm contains hardly any blood cells. The connective tissue of the walls is infiltrated with some erythrocytes, mononuclear cells, and a few pigmented leucocytes.

The seventh cyst is small and in the submucosa, but it involves some of the fibres of the internal muscular coat. Mononucleated leucocytes and degenerated erythrocytes, with a section of a worm containing some pigmented cells and a few degenerated erythrocytes, are found in the cavity of the cyst. Two thin incomplete bands of connective tissue practically subdivide the cavity into three little chambers which communicate with one another. The walls show a moderate infiltration of mononuclears, small round cells and a great number of eosinophiles. Small rows of pigmented cells cross the muscular coats and are collected in little clumps in the subperitoneal space.

It is evident that the fourth cyst was infected from the ulcer of the mucous membrane, and that the necrosis extended to the third cyst.

COLON

Section through thickened wall of colon at level of fold of mucous membrane.

Cross-section of the wall shows two small cysts in the submucosa, under the fold of mucous membrane. Their contents are purely haemorrhagic, and only comprise erythrocytes and a few mononuclears with a section of a worm in each cyst. In the internal muscular coat is a long narrow cyst with dense fibrous cells. The contents of the cyst are degenerated red blood cells, pigment granules, and several giant cells containing considerable pigment; a cross-section of a worm is seen with a few fibrous-looking cells in its intestine. The cyst is confined to the internal muscular coat. A small cyst is situated in the external coat and extends to the subperitoneum. The intermuscular space is infiltrated by mononuclear leucocytes and a few eosinophiles. The contents of this cyst are mononuclears and degenerated erythrocytes, with a small section of a worm; no giant or pigment cells are seen, but there is a considerable number of eosinophiles. Near this cyst, but apparently developed in the subperitoneum, is a fibrous enclosed cyst. The cavity contains the remains of degenerated red blood cells and a few polymorphonuclear cells. No worm is found. Strands of apparently new connective tissue cross the cyst. A calcified area is present at one end. The walls contain some mononuclear leucocytes, and a few giant cells are in the side adjoining the internal muscular coat. The outer peritoneal covering is covered with layers of fibrous exudate. No bacilli are found in the cyst, but in the outer layers of the exudate a few small bacilli are present.

Cysts invading the ileo-caecal valve.

A small nodule was sectioned, and found to consist of a submucosal cyst which had apparently ruptured and allowed the escape of the worm. The contents were scanty and comprised a few mononuclears with a number of small round cells. Much new connective tissue was present in the cyst cavity. No bacilli were found. Giant cells were present in the submucosal tissue immediately beyond the wall of the cyst.

Tumour developing in the base of the valve segment. It comprises two small cysts, each containing cross sections of a worm, which distend the submucosal space and press upon the muscularis mucosae. Pigmented leucocytes are very numerous, but no giant cells are seen. The infiltration of all three cysts is slight, and consists of large mononuclears, small lymphocytes and a few eosinophiles. The vessels in the neighbourhood are full of normal looking red blood cells and mononuclears.

Various other nodules in the caecum and colon were sectioned and found to have the characters already noted in nodules occurring in the ileum. Several submucosal cysts were examined which were immediately below small ulcerations of the mucous membrane. The tissue of the mucous membrane was seriously degenerated, the area was partially denuded of cells and the lymph follicles swollen, but the cyst walls were not attacked so long as the muscularis mucosae remained intact. In one case the necrosis affected the outer half of the adjacent cyst wall.

Examination of the extensive black patches occurring in the wall of the gut has been unsatisfactory. Some of the patches are situated in the submucosa, others are in the subperitoneal space. The patches are caused by extravasated blood, and many well-preserved erythrocytes are still present; much blood pigment has been soaked up by the surrounding tissues, while only a moderate number of macrophages contain pigment. One or two patches in the subperitoneum, in the vicinity of cysts were invaded by large giant cells, many of which contained pigment. Evidences of repair were present in all the extensive areas. Iron pigment was rarely demonstrable. No traces of œsophagostome larvae or ova have been found.

Appendix vermiformis. Subperitoneal tumour, sectioned transversely.

This large cyst has developed in the subperitoneal space of the wall of the appendix, and from there has extended outwards into a cyst-mass larger than the appendix. The external muscular coat of the appendix is not involved. Between the external muscular coat and the tumour, the large blood vessels are seen cut across; these are filled with red blood cells and some leucocytes. Other blood vessels follow the peritoneal fascia along the tumour wall, penetrating to the interior of the cyst. The walls of the cyst consist of well organized connective tissue, and from the main walls thick strands cross the cavity and divide it into two main parts. A study of serial sections proves that the cyst tumour consists of an internal or proximal and an external or distal cyst. They are separated by the main divisional wall, which is continued completely across, from the upper to the lower walls of the tumour. These cysts are subdivided into small chambers by incomplete side walls of connective tissue. In one of the chambers of the distal cyst two transverse sections of a worm are seen; in other slides longitudinal sections of the cephalic end of the worm are found in the larger chamber of the external cyst. The serial sections show that the proximal cyst contains no worm.

The contents of the cyst are mainly leucocytes and red cells. Great numbers of polymorphonuclears are present in the cyst chambers, and the partition walls are infiltrated by mononuclears.

Higher magnification. The contents are nearly all pus cells with a few well-preserved and many degenerated erythrocytes, a few mononuclears, free pigment, and granular débris. The outer walls of the proximal cyst are lined with well-preserved pavement epithelium. The connective tissue is infiltrated by masses of mononuclears and some small round cells. Very few eosinophiles are seen. The blood vessels are large, and the veins are filled with many polymorphonuclear and mononuclear leucocytes, and well preserved erythrocytes. No bacilli or cocci are to be found. The sections of the worm show that the intestine contains some normal-looking and degenerated red blood cells. Large pigmented cells are found in the proximal cyst, and a few giant cells are in the wall.

The spleen sections show a very marked hyperplasia of the connective tissue of the capsule in which are numerous capillaries full of erythrocytes and some mononucleated leucocytes; in places the fibres are undergoing hyaline degeneration. The fibres of the upper strata are infiltrated with mononuclear and polymorphonuclear leucocytes; the lower strata of the fibrous mass is not so much infiltrated. Golden brown to black pigment is scattered throughout the spleen in small and large clumps. It is especially marked about the blood vessels in the trabeculae and in the centre of the follicles. Many lymphoid cells appear to be degenerated; large mononuclear cells are present in the sinuses and lymphatics. An overgrowth of fibrous tissue has occurred in the tissue near the capsule. Many cells stain badly, but this may be due to decomposition. This is especially the case with the liver and kidneys.

The liver exhibits slight interstitial changes; small round cells are collected around the portal sheaths, golden brown to blackish pigment is deposited in some of the peripheral cells of the lobules and in the interspaces. The organ shows extensive decomposition, and large bacilli are present.

The kidneys evidence cloudy swelling, and the vessels are filled with blood The organs are too decomposed to admit of more exact determination of the changes present.

The heart muscle is degenerated and the fibres show some fatty degeneration The muscle of the left ventricle is hypertrophied, and contains some brownish blac pigment granules, which do not give the iron reaction. Around the aortic ri is a pronounced area of old atheromatous changes, and one or two of the plaques are calcified.

The mesenteric glands show hyperplasia of the connective tissue reticulum the lymphoid cells are increased, and large mononuclear and multinucleated cells are present. Pigment granules are found, but not in any great number Eosinophiles are especially numerous in the small retro caecal glands. In some of the glands near the diseased bowel, spots of necrosis are found; the lymphoid cells stain badly, and are very granular; polymorphonuclear leucocytes are accumulated in the lymph spaces.

The Brazilian case of œsophagostomiasis corresponds to the cases of the disease, occurring in apes and monkeys, described by Weinberg In certain important points the case in man differs. The lesions are more extensive in the Brazilian case; more tumours are formed, and greater damage appears to have been caused to the wall of the bowel The œsophagostome larvae have developed in the small intestine of the man, an unusual occurrence, as Weinberg has not recorded such a situation, and it was believed that the cysts of œsophagostomes were limited by the ileo-caecal valve. It is probable that the cysts in the ileum are of more recent development than those in the caecum and colon.

Weinberg records the presence of an adult female œsophagostome and of spherical eggs measuring 52 μ, in the cyst cavity of a nodule taken from the caecum of a chimpanzee, and, in another case, he has found minute larvae in the muscular coat. In the Brazilian case I have not as yet succeeded in discovering either a mature worm or the ovum. More tumours are still being sectioned in the hopes of finding an adult œsophagostome, which would help to explain the presence of the small sections of larvae in the submucosa, subperitoneum and intermuscular spaces.

The data obtained from examination of twelve nodules of the ileum, eleven of the caecum, three of the ileo-caecal valve, one of appendix vermiformis, and nine of the ascending colon allow of the following conclusions being drawn:—

1. The œsophagostome larvae,* as stated by Weinberg, reach the bowel by way of the blood. The larvae rupture a small blood vessel and become encysted in the submucosa, in the internal or external muscular coats of the bowel or in the subperitoneal space. In no case has a cyst been found above the level of the muscularis mucosae. The favourite sites for the development of the cysts appear to be in the submucosa and external muscular coat with invasion of the subperitoneal space. Cysts developing primarily in the internal muscular layer are not uncommon. The subperitoneal space appears to be a favourite site for the cysts of the ileum, but in the caecum and colon it is extremely rare to find a subperitoneal cyst. Nearly all the cysts have developed in the outer fibres of the external muscular coat and have immediately extended to the looser tissues of the subperitoneal space. In no case has a cyst been found to contain more than one worm.

2. The worm can moult and become an immature œsophagostome and leave its cyst cavity. Weinberg has found them in the lumen of the intestines. An empty cyst can become cicatrized or calcified. Acute inflammation of a cyst does not affect the larva.

Necrosis of one cyst can extend to another one lying in the immediate vicinity. The amount of infiltration around a cyst depends to a certain extent on the amount of irritation produced by the worm. Weinberg has shown that the œsophagostome can produce haematoxic substances analagous to those secreted by the sclerostome of the horse, and it is probable that this affords an explanation of the number of eosinophiles observed around some cysts and not around others, although they may be lying close at hand. The infiltration can be induced by bacilli entering the cyst by way of the blood stream, or by ulceration of the distended mucous membrane.

The haemorrhagic contents of the cysts and the degeneration of the blood cells cause the formation of iron pigment, which can be taken up by large leucocytes and conveyed to the subperitoneum. Iron pigment has been found in large quantities in the spleen and, to a less extent, in the liver and mesenteric glands.

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* The larvae obtain nourishment from their host. Many erythrocytes have been found in the lumen of the intestine of a worm. Pus cells and bacilli are frequently found if the contents of the cyst are septic.

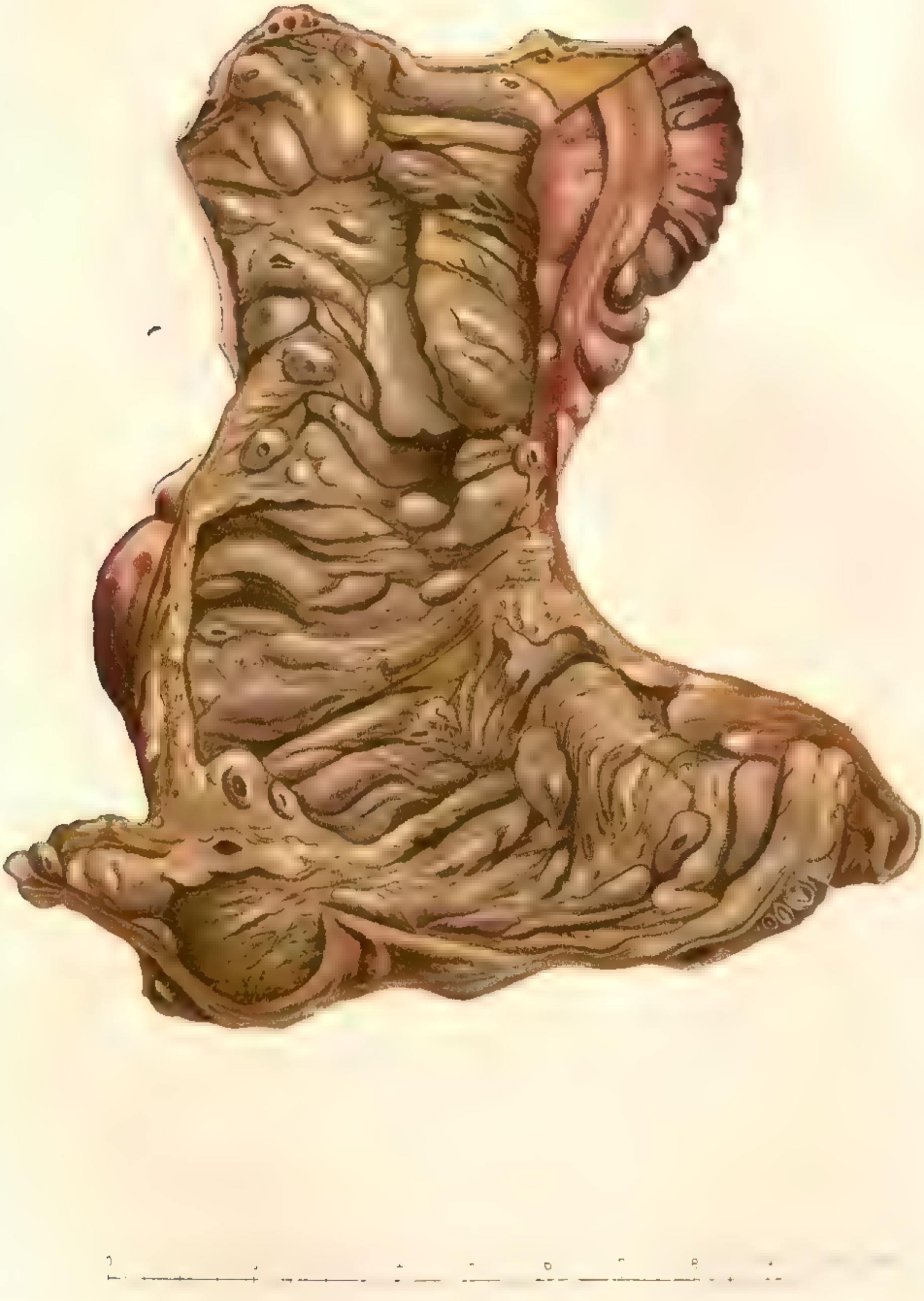
PLATE I.

Caecum and ascending colon. Along the longitudinal muscular band some of the raised, dark, transverse, haemorrhagic, sub-peritoneal cysts can be seen, also two small oval ones. Large tumour masses are developed in the submucosa, many of them have been opened up. Note the transverse arrangement of the cysts. The raised ochre-coloured patches are foci of necroses occurring in the cyst walls, and the surrounding mucous membrane is inflamed and ulcerated. A large submucosal tumour is seen in the lower third of the plate. (Compare Plate III, fig. 2). The cut surface of the cross-sections gives a good idea of the amount of infiltration caused by these cysts, and the honey-combed condition of the wall about the longitudinal muscular band is especially well seen in the lower portion of the drawing. (Compare Plate V, figs. 1, 2.)

[illegible] stomum var. Thomas

PLATE I.

[illegible] Along the longitudinal muscular [illegible] [illegible] cysts can be seen, also two small oval [illegible] many of them have been [illegible]ed up. Note the transverse arrangement of the cy[illegible]. The raised ochre-coloured patches are foci of [illegible] occurring in the cyst wall and the surrounding [illegible] membrane is inflamed and ulcerated. A la[illegible] tumour is seen in the lower third of the [illegible] Plate III, fig. 2). The cut surface of [illegible] gives a good idea of the [illegible] by these cysts, and the [illegible] the wall about the longi[illegible] [illegible]ally well seen in the lower [illegible] Compare Plate V, figs. 1, 2.



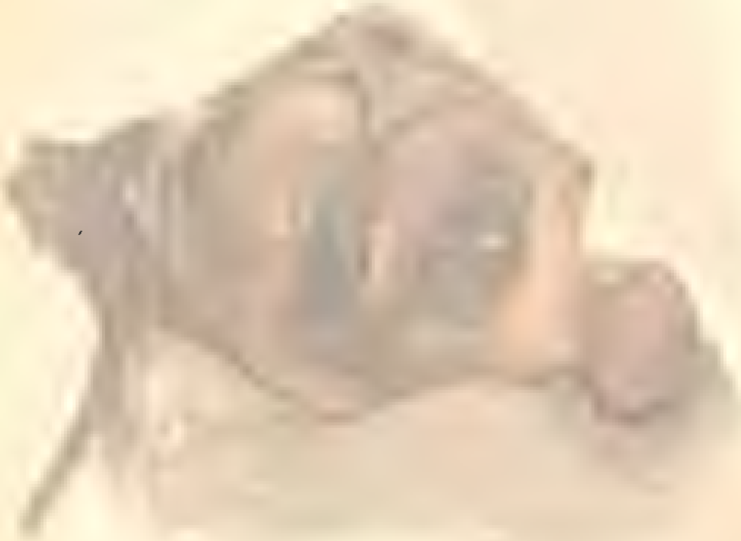
Œsophagostomum stephanostomum var. Thomasi

PLATE II.

Fig. 1. *Caecum and appendix.* External surface. The gut was held up to the light in order to show more clearly the extent of the black haemorrhagic patches. Note the black areas at the apex. These are masses of subperitoneal tumours infiltrating a longitudinal muscular band. Some necrotic and calcareous patches are seen coloured yellow. *Appendix vermiformis.* Probe passed through lumen into caecum. Notice tumour mass on wall of caecum; it is composed of a number of submucosal and subperitoneal cysts.

Fig. 2. Compare Plate III, fig. 1.

Interior of Caecum. Edges of bowel held apart, and tumour masses depicted by transmitted light, so as to show the thickening, and the cord-like appearance of the cysts. These masses are composed of a number of submucous, haemorrhagic cysts near each other. Note the general tumour development at the upper and lower portions of the drawing; these correspond to the clusters of submucosal and subperitoneal cysts about the longitudinal muscular bands. Probe passed through appendix.



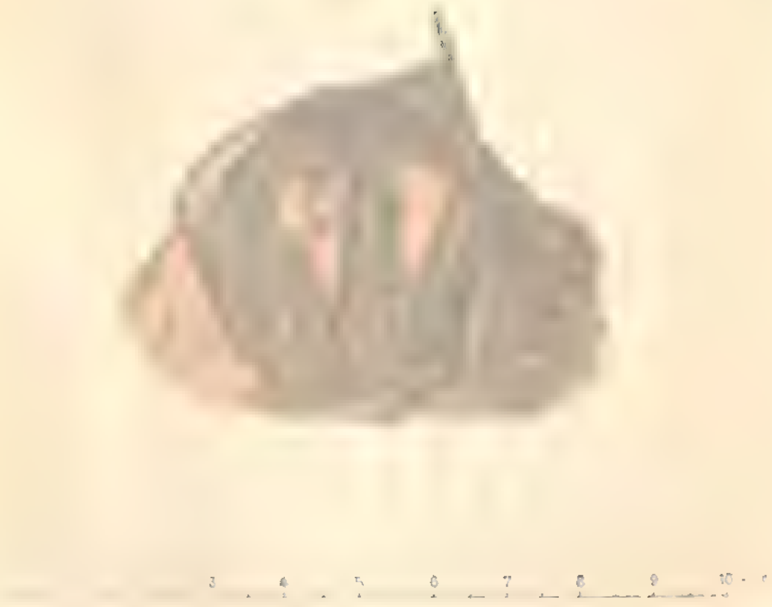

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PLATE II

Fig. 1. *Caecum and appendix.* External surface. The g held up to the light in order to show more clearly extent of the black haemorrhagic patches. Note the [illegible] is at the apex. These are masses of subper tumours infiltrating a longitudinal muscular band. S necrotic and calcareous patches are seen coloured ye *Appendix vermiformis.* Probe passed through into caecum. Notice tumour mass on wall of caecum, is composed of a number of submucosal and subperit cysts.

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Interior of caecum. Edges of bowel held apart a [illegible] by transmitted light, so as to sh [illegible] the cord-like appearance of the cyst [illegible] composed of a number of su [illegible] near each other. Note the ge [illegible] at the upper and lower portions of [illegible] these correspond to the clusters of su [illegible] subperitoneal cysts about the longitu [illegible] Probe passed through appendix.

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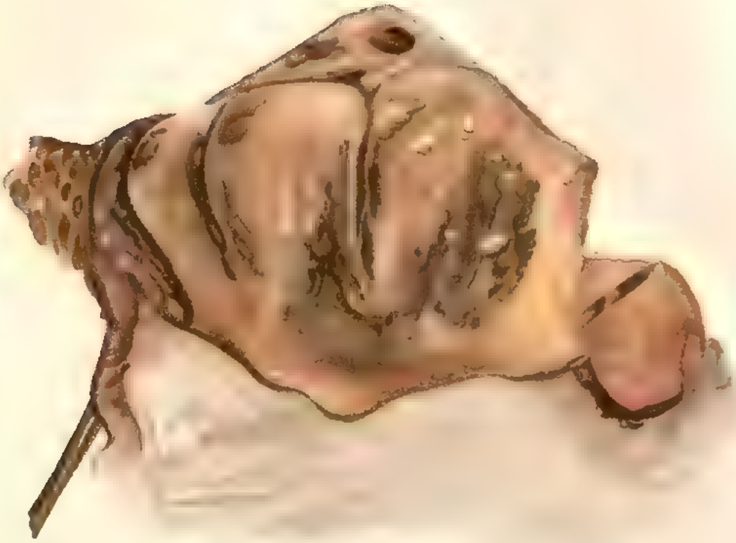

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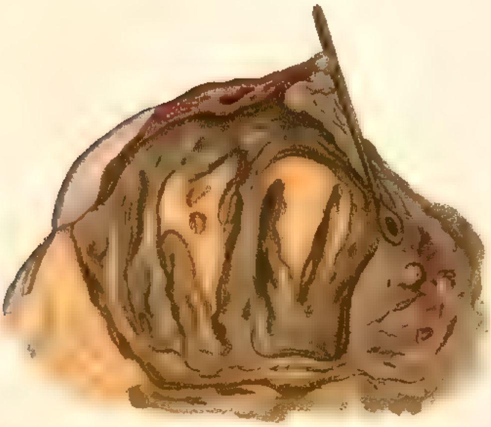

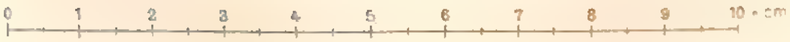

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Werner u. Winter

Œsophagostomum stephanostomum var. Thomasi

Plate III.

Fig. 1. *Caecum.* The cord-like, twisted masses of cysts are al merging into one broad area (here it is the anterior longitudinal band). Note the outline of a worm showing through the greatly thinned mucous membrane. The thickening of the wall is well seen. (Compare Plate II fig. 2.)

Fig. 2. *Interior of colon.* A number of submucous cysts are seen In the upper left-hand corner of the drawing is a large cyst, (in Plate I, seen in lower third of drawing), the size of a large bean. Note the necrotic patches in some of the cyst walls.

[illegible]

PLATE III.

[illegible] *Caecum.* The cord-like, twisted masses of cysts are merging into one broad area (here it is the anterior l itudinal band). Note the outline of a worm sh through the greatly thinned mucous membrane. [illegible]

[illegible] [illegible] Note the necrotic patches in some of cyst w [illegible]

Plate III

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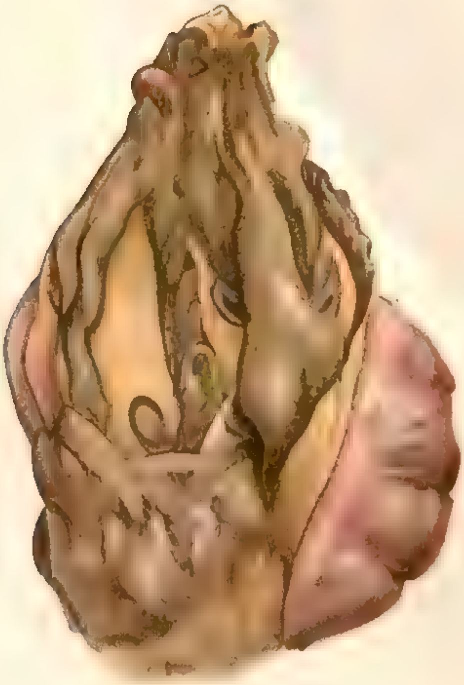

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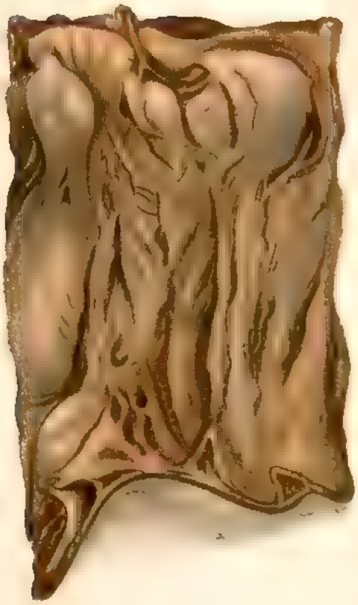

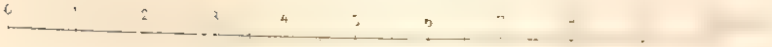

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Œsophagostomum stephanostomum var. [illegible].

PLATE IV.

Fig. 1. *External surface of ileum.* The large leech-like masses are subperitoneal haemorrhagic cysts. Each mass is composed of one cyst.

Fig. 2. *External and part of internal surface of ileum* Portio of intestine showing small oval and leech-like cysts. Cys opened and worm partially withdrawn. (Compare fig 3 To the extreme right of drawing is a triangular piece o the hypertrophied external muscular and peritoneal coats of the bowel, and from the end are seen projecting portions of two worms. (Compare fig. 4.) Below this is a long cylindrical submucosal cyst.

Fig. 3. Small oval cyst as depicted in fig. 2. × 8.

Fig. 4. Hypertrophied external muscular and peritoneal coats showing the ends of two worms as depicted in fig. 2 ×

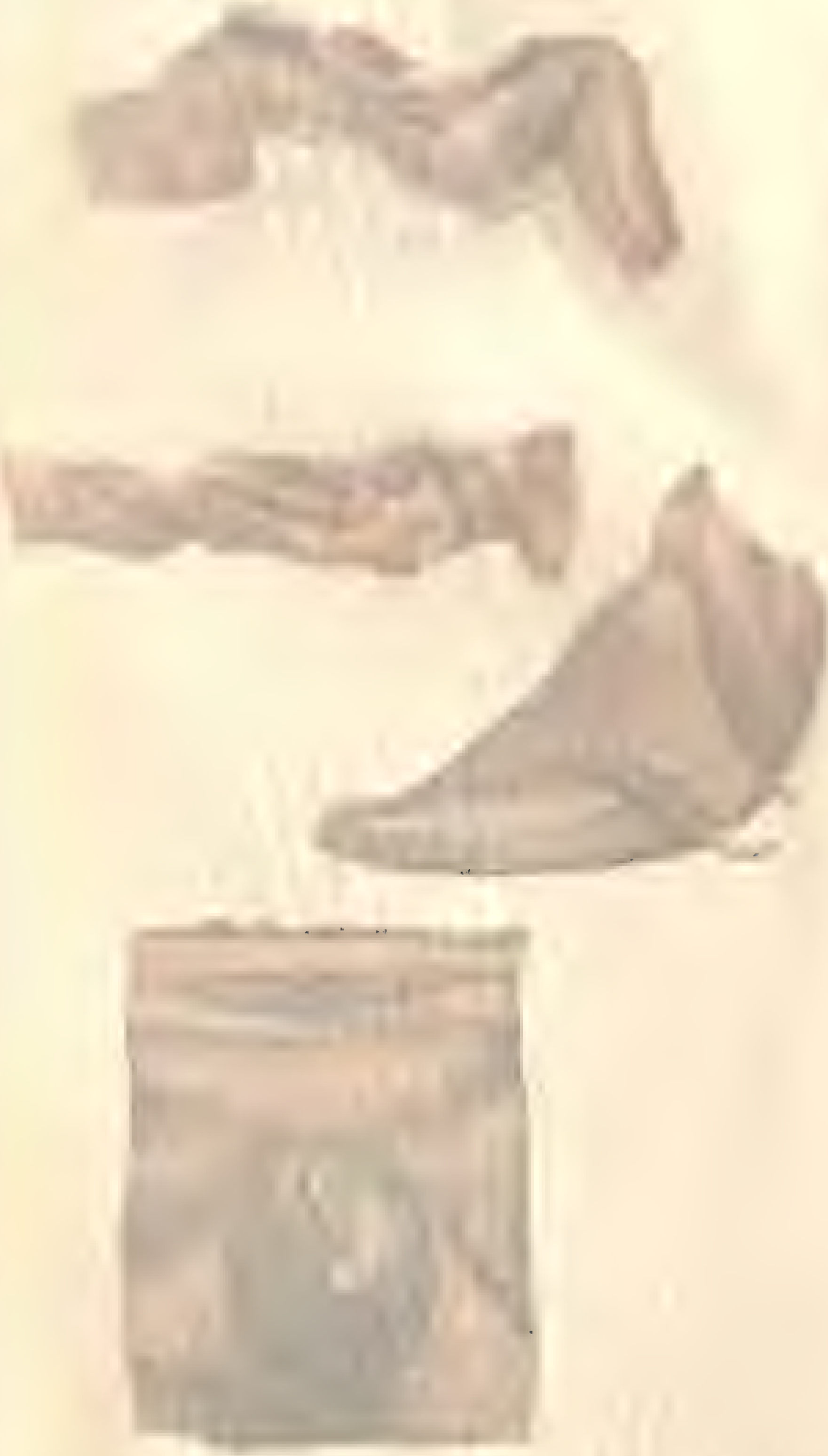

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PLATE IV.

[illegible] surface of ileum. The large leech like [illegible] [illegible] haemorrhagic cysts. Each [illegible] [illegible] of one cyst.

External and part of internal surface of ileum. [illegible] of intestine showing small oval and leech like [illegible] opened and worm partially withdrawn. ([illegible] To the extreme right of drawing is a triang[illegible] the hypertrophied external muscular and per[illegible] [illegible] the bowel, and from the end are seen proj[illegible] [illegible] two worms. (Compare fig. 4) Below [illegible] [illegible]lindrical submucosal cyst

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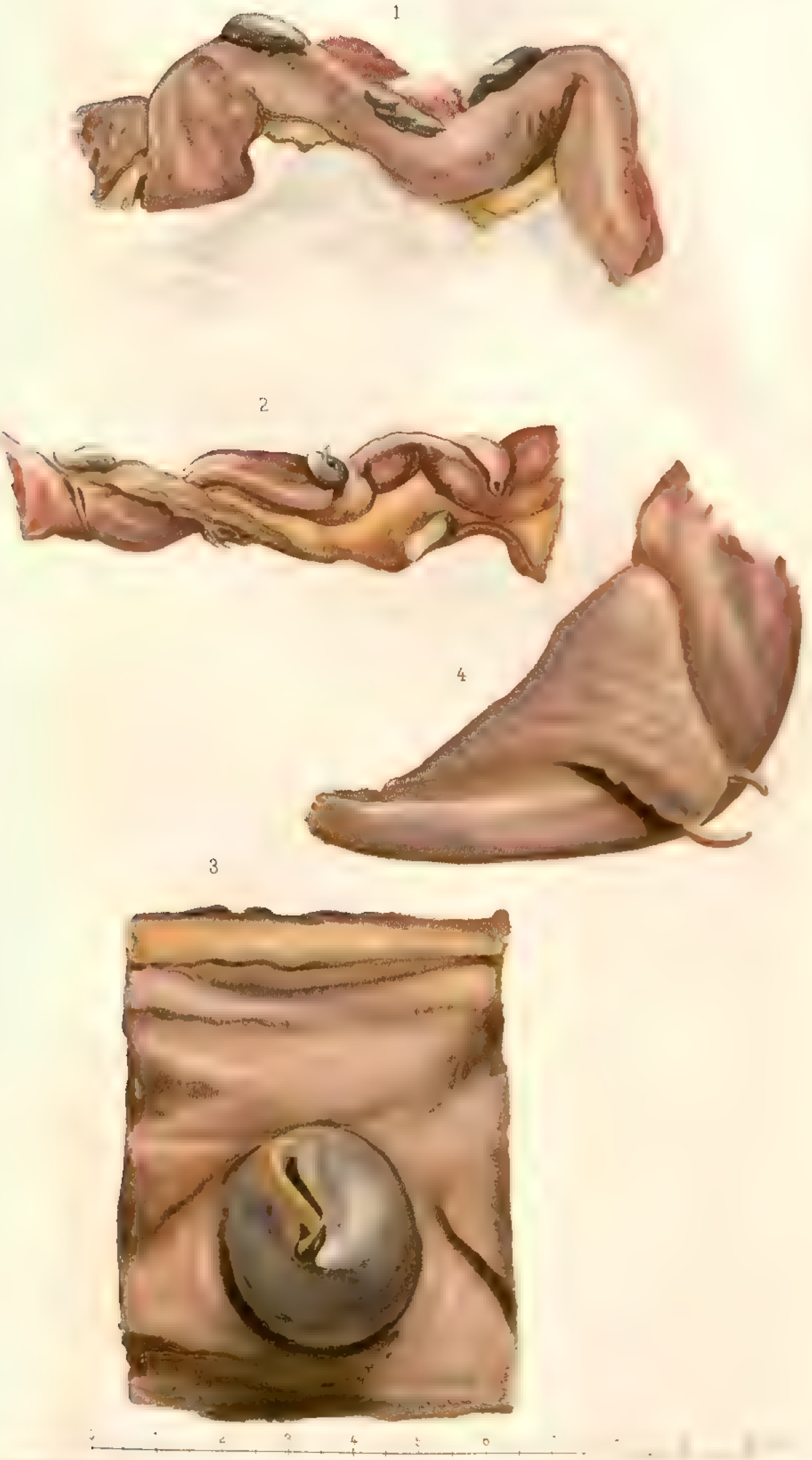

Œsophagostomum stephanostomum var. [illegible]

PLATE V.

Fig. 1. *Cross sections of wall of colon and caecum.* Infiltration and honeycombed condition produced by submucosal, subperitoneal or intra-muscular cysts. Note the size of some of the cyst cavities, and the number of cysts in a small piece of bowel wall. The hyperplasia of the fibrous tissue is well seen and resembles the rings of a tree-trunk.

Fig. 2. *Wall of caecum.* Thickening of wall by fibrous tissue. This cyst was greatly inflamed.

Fig. 3. *Ileum.* Two small nodular subperitoneal cysts. They do not communicate with one another.

Fig. 4. *Small omental gland*, showing œsophagostome partially withdrawn. It had penetrated the gland and become encysted. × 12.

Fig. 5. *Ileum.* Two small, flat, button-shaped subperitoneal cysts. The cysts are quite separate.

Fig. 6. *Ileum.* Note subperitoneal cyst on side of mesenteric attachment. It is attached to the side wall and, with two small submucosal cysts, has produced a puckering of the wall of the intestine. Several of these cysts produced a sacculated condition of the tube.

Fig. 7. *Œsophagostome* lying on external surface of colon. The gastro epiploica, by which it was covered, is turned back. Note three small yellowish points near the worm; these are parts of worms in other cysts. The membranous coverings of the cysts were very thin, and were torn in drawing back the pad of fat. The small dark round spot is the hole made by the worm escaping from its cyst. × 16.

Fig. 8. *Colon.* Œsophagostome withdrawn from its cyst cavity. × 20.

Fig. 9. *Colon.* Two cysts magnified to show their plastic form. × 16.

Fig. 10. *Œsophagostome.* It had ruptured the walls of its subperitoneal cyst and had penetrated the mesenteric attachment of the ileum. × 12.

PLATE V

[illegible] *sections of wall of colon and caecum* [illegible] [illegible] honeycombed condition produced by submucosal peritoneal or intra-muscular cysts Note the size of the cyst cavities, and the number of cysts in a piece of bowel wall The hyperplasia of the fibrous is well seen and resembles the rings of a tree trunk

Fig 2. *Wall of* [illegible] Thickening of wall by fibrous The [illegible] [illegible]ly inflamed

Fig 3 *Ileum* [illegible] nodular subperitoneal cysts The [illegible] with one another.

Fig 4 [illegible] *gland*, showing oesophagostome [illegible] [illegible] It had penetrated the gland and be[illegible] [illegible] × 12.

Fig [illegible] [illegible] Two small, flat, button shaped subperitonea[illegible] The cysts are quite separate

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[illegible] [illegible] on external surface of colon. [illegible] which it was covered, is turned [illegible] Note the [illegible] yellowish points near the worm, [illegible] are [illegible] in other cysts The memb[illegible]

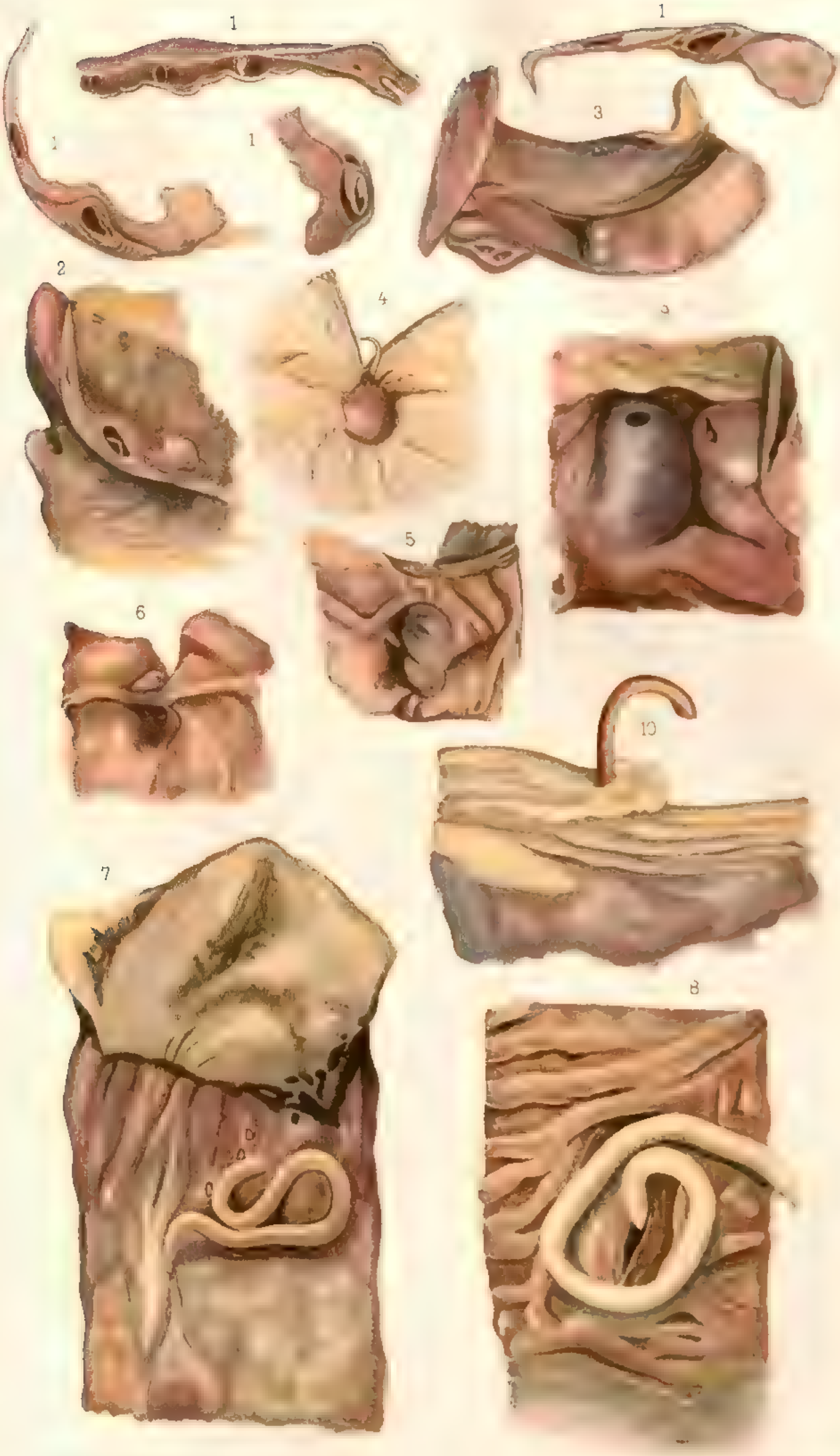
[illegible] made by the worm escaping from its

[illegible] Œsophagostome withdrawn from its cyst

Fig 9. *Colon.* Two cysts magnified to show their plastic [illegible]

Fig 10. *Œsophagostome.* It had ruptured the walls of its sub-peritoneal cyst and had penetrated the mesenteric attachment of the ileum. × 12

Plate V



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Œsophagostomum stephanostomum var. Thomasi

PLATE V^{A}

Portion of ileum showing leech-like subperitoneal cy Longitudinal section. In the degenerated haemorrhag contents two sections of a worm are lying. Note the collection of leucocytes around the inside of the wall of the cyst and the leucocytic infiltration of the walls. In the internal muscular coat is a small cyst. The leucocyt infiltration is extensive and extends into the submucosa

PLATE Va.

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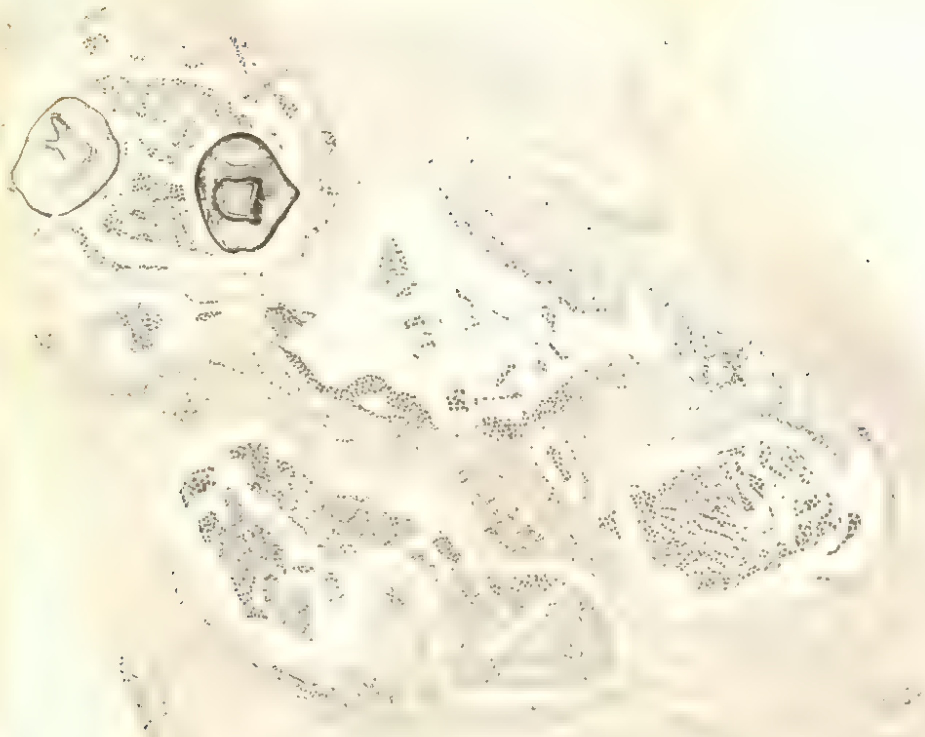
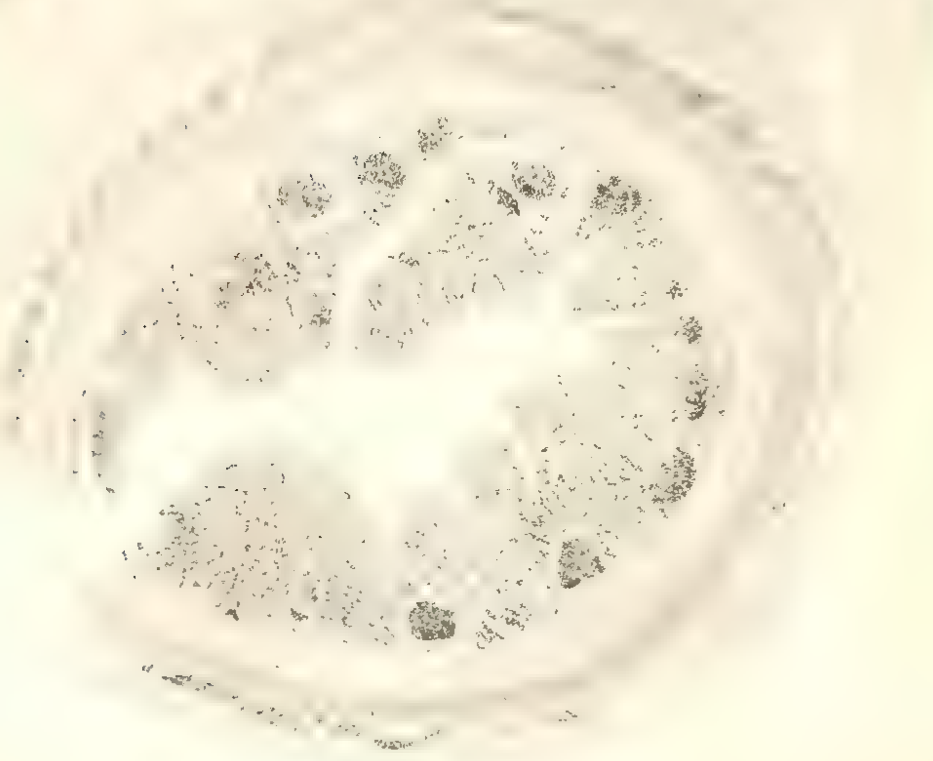
PLATE V^{B}

Subperitoneal tumour growing from the wall of the appendix vermiformis. Note the division of the tumour into two cysts and the partial sub-division of them by bands of connective tissue. The contents of cysts are chiefly pus cells Two cross-sections of an œsophagostome are seen in the outer-cyst.





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ÉTUDE ZOOLOGIQUE DE L'ŒSOPHAGOSTOME DE THOMAS

PAR

A. RAILLIET, PROFESSEUR

ET

A. HENRY, CHEF DE TRAVAUX

À L'ÉCOLE NATIONALE VÉTÉRINAIRE D'ALFORT

(Received for publication March 10th, 1910)

(Plate VI)

L'examen attentif des exemplaires du Nématode que nous a soumis M. le Dr. H. Wolferstan Thomas nous a permis de relever les caractères suivants :—

Le corps est cylindroide, épais, atténué seulement vers les extrémités. Le tégument est strié en travers ; les stries sont espacées de 27µ au niveau de la terminaison de l'œsophage.

L'extrémité antérieure offre une brève saillie cuticulaire (*bourrelet péristomique*), en arrière de laquelle apparaît un second renflement beaucoup plus ample (*vésicule céphalique*). Cette vésicule s'atténue progressivement en arrière ; mais, dans la région ventrale, elle s'arrête brusquement au niveau d'une dépression transversale (*fente ventrale*) dans laquelle vient déboucher le canal excréteur du système aquifère. Un peu en avant de la dépression dont il s'agit, laquelle est située à 360-365µ de l'extrémité antérieure, la vésicule céphalique offre un faible rétrécissement.

A 500-580µ de l'extrémité antérieure, existent deux papilles latérales (*papilles cervicales*) légérement asymétriques, à base hémisphérique et à pointe subulée.

La marge de la bouche est garnie de 38 lamelles (*coronule externe*), dont l'extrémité libre est semi-circulaire ; immédiatement en arrière de ces lamelles se montrent en nombre double d'autres languettes plus courtes, fortement chitinisées et réfringentes, qu'on peut regarder comme représentant une *coronule interne*.

La bouche donne entrée dans une capsule buccale très surbaissée d'une hauteur moyenne de 22μ, un peu déprimée en avant sur la ligne médiane dorsale, à la terminaison du court tunnel qui protège le canal excréteur de la glande œsophagienne. La capsule est à section ellipsoïde ; elle mesure environ 115μ dans son diamètre dorso-ventral et 95μ dans son diamètre latéral.

Il existe six papilles céphaliques traversant le bourrelet péristomique : deux latérales à base large et à pointe mousse, et quatre submédianes plus longues, plus aiguës, à base cylindrique.

L'œsophage, renflé en massue, est long de 1 mm. 100 à 1 mm 200, sur une largeur maxima de 225 à 275μ atteinte vers le sixième postérieur ; à son extrémité antérieure, sa lumière s'évase en un large entonnoir occupé en grande partie par des replis du revêtement chitineux qui constituent six grosses dents mousses.

Le *mâle* est long de 17 à 22 mm, épais d'environ 750μ. Il est pourvu d'une bourse caudale formée de deux lobes latéraux très amples réunis par un petit lobe médian legérement échancré. Les côtes sont épaisses et massives ; les antérieures et les moyennes sont fendues, les postérieures et les postérieures externes naissent d'un tronc commun, les postérieures portent un rameau latéral court, incurvé en dedans et inséré vers leur tiers supérieur. Deux papilles prébursales. Deux spicules égaux, mesurant 1 mm. 380 à 1 mm. 475, à extrémité libre légérement arquée en faucille.

La *femelle* est longue de 16 à 20 mm., épaisse d'environ 900μ vers le milieu de la longueur. L'extrémité caudale est brusquement atténuée ; elle est terminée par un petit appendice conique parfois retroussé vers la face dorsale, et portant deux petites papilles latérales qui sont probablement les homologues des papilles prébursales du mâle. L'anus est situé à 230μ environ de l'extrémité postérieure, la vulve à 500-525μ ; ces deux orifices ne donnent lieu à aucune saillie tégumentaire.

Le vagin, après un court trajet en avant, aboutit aux ovéjecteurs, qui sont très courts et opposés, formant dans leur ensemble une poche longitudinale oblongue dont les deux extrémités sont renflées chacune en un puissant bulbe globuleux bien distinct. A chaque bulbe fait suite la partie non musculeuse de l'utérus auquel il appartient : l'utérus antérieur se dirige directement en avant, l'autre décrit une boucle très courte pour prendre la direction du premier.

Aucune des femelles examinées ne contenait d'œufs ; il s'agissait donc de formes immatures.

Les caractères qui précèdent permettent de classer, sans hésitation, ce parasite dans le genre *Œsophagostomum* Molin, 1861 de la famille des *Strongylidæ*, sous-famille des *Strongylinæ*, section des *Œsophagostomeæ*. Ce genre est caractérisé par sa capsule buccale très surbaissée, souvent même réduite à un simple anneau chitineux, par sa marge buccale pourvue de lamelles formant une coronule, par la présence habituelle d'une vésicule céphalique qui s'arrête brusquement sur la face inférieure au niveau de la fente ventrale.

Reste à en déterminer l'espèce. Or, l'étude comparative des formes connues conduit à écarter délibérément celles dont les coronules ne comprennent qu'un petit nombre de lamelles, comme l'*Œsophagostomum brumpti* Raill. et Henry, 1905, qui est également parasite de l'Homme.

Le rapprochement s'impose, au contraire, avec les formes possédant des coronules à lamelles nombreuses. L'une de celles ci a tout d'abord attiré notre attention : c'est l'*Œsophagostomum dentigerum* Raill. et Henry, 1906, du Chimpanzé, dont les dimensions, l'armature œsophagienne et la longueur des spicules concordent, d'une façon frappante, avec les particularités relevées dans le parasite brésilien.

Nous soupçonnions, d'ailleurs, une semblable parenté entre celui-ci et l'*Œsophagostomum stephanostomum* Stossich, 1904, du Gorille. La description trop sommaire de Stossich ne permettait malheureusement pas de se prononcer sur ce point. Mais, grâce à l'obligeance des professeurs Shipley et Leiper, nous avons pu examiner les exemplaires types de l'espèce et reconnaître que nos prévisions étaient fondées.

De sorte que les parasites de l'Homme, du Chimpanzé et du Gorille nous sont apparus comme de simples variétés d'une seule et même espèce.

Les différences que nous avons pu relever entre ces trois formes sont, en effet, de faible importance. Celles qui nous ont paru constantes résident surtout dans la bourse caudale des mâles.

Chez l' *Œs. stephanostomum* type, le rameau externe de la côte postérieure s'insère sensiblement au quart supérieur de la partie libre de cette côte et se porte directement en dehors.

Dans la var. *dentigera*, ce rameau s'insère au tiers supérieur et se recourbe en dedans pour suivre une direction parallèle à celle du rameau principal.

Dans la variété brésilienne, il s'insère au tiers supérieur et se recourbe en dedans pour se rapprocher du rameau principal. Il semble, enfin, que l'extrémité caudale de la femelle subisse ici un rétrécissement beaucoup plus brusque que dans les deux formes précédentes. Nous avons proposé de dénommer ce nouveau parasite de l'Homme *Œs. stephanostomum* var. *Thomasi*. Si l'on est amené à lui attribuer un rang spécifique, il deviendra naturellement *Œs. Thomasi*.

Nous ferons remarquer, en terminant, que la parenté de l'Œsophagostome humain avec ceux du Chimpanzé et du Gorille tendrait à lui assigner une origine africaine.

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EXPLICATION DE LA PLANCHE

Fig. 1.—Mâle, grandeur naturelle.

Fig. 2.—Femelle, grandeur naturelle.

Fig. 3.—Extrémité antérieure d'une femelle, vue par la face ventrale. × 55.

Fig. 4.—Extrémité antérieure d'une femelle, vue par la face dorsale × 225.

Fig. 5. -Extémité antérieure d'un mâle, vue de face. × 225.

Fig. 6.—Extrémité caudale du mâle, vue de côté. × 20.

Fig. 7.—Extrémité caudale de la femelle, vue de côté. × 20.

Fig. 8.—Côtes postérieures de la bourse caudale chez l'*Œsophag* *stomum thomasi* de l'Homme. × 150.

Fig. 9.—Côtes postérieures de la bourse caudale chez l'*Œs. dentigerum* du Chimpanzé. × 150.

Fig. 10.- Côtes postérieures de la bourse caudale chez l'*Œs* *stephanostomum* du Gorille. × 150.

Œsophagostomum stephanostomum var. Thomasi

[illegible]ATION DE LA PLANCHE

[illegible] grandeur naturelle.

[illegible] antérieure d'une femelle, vue par la face [illegible]

[illegible] Extrémité antérieure d'une femelle, vue par la face [illegible] × 225.

5.—Extémité antérieure d'un mâle, vue de face. × [illegible]

[illegible] 6.—Extrémité caudale du mâle, vue de côté. × 2[illegible]

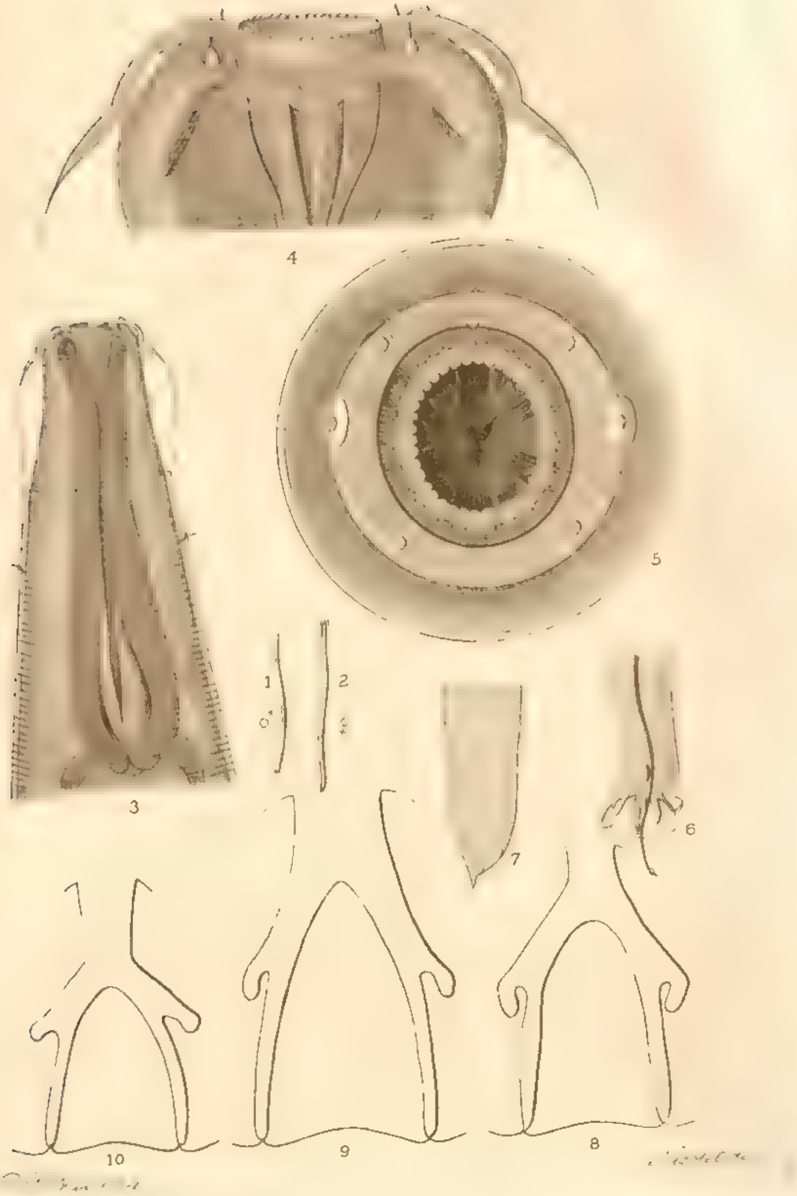
[illegible] 7.—Extrémité caudale de la femelle, vue de côté. × 2[illegible]

[illegible] de l'Homme. × 15[illegible]

[illegible] 9 [illegible] postérieures de la bourse caudale chez [illegible] [illegible]né. × 150.

[illegible] Gorille. × 150.

PLATE VI.



Œsophagostomum stephanostomum var. Thomasi

"MOSSY" FOOT OF THE AMAZON REGION, AN INFECTIVE VERRUCOTIC CONDITION AFFECTING THE SKIN OF THE UPPER AND LOWER LIMBS

BY

H. WOLFERSTAN THOMAS, M.D., C.M. (McGILL).

Expedition to the Amazon, 1905-1909.

From the Research Laboratory in Manáos, State of Amazonas, North Brazil.

(Plates VII, VIII, IX)

My attention was attracted to a verrucoid condition usually affecting the lower limbs of natives of the Amazon region. It appears to be auto-infective, and nearly all the cases exhibited one or more infective foci. Many patients complain of the exquisite tenderness of the papillomata, and on account of the vascularity even slight injuries may cause pain and bleeding. The condition is of slow growth. It is probable that the pain and bleeding are the chief reasons which induce the patient to ask for treatment.

The photographs* (Plates VII, VIII) depict a severe type of the infection. The patient, a man aet. 23, an Amazonense of the peasant class, claimed that he had injured his right foot about nineteen months previously. The foot remained tender and swollen. He does not remember having had any fever other than sezões (ague), and about a year later he noticed small vesicles appearing on the foot, on the outside of the heel. The vesicles were moist and persisted for a couple of weeks, when they gave place to small dry, warty growths, which gradually advanced and spread to the toes, around the heel and the inside of the foot. The condition had persisted for seven months before the photographs were taken.

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* I am indebted to Messrs. G. Huebner and Amaral, of Manáos, for the photographs of the case.

The whole of the right leg from below the knee is swollen and hard, pitting on strong pressure ; the skin is tense, smooth, and glossy, and through the hair the superficial lymphatics and small vessels can be seen. Below the knee, on the outside, is a small, hard fibrous nodule the size of a large pea. The foot is greatly enlarged and a deep sulcus is formed by a fold at the back of the ankle joint. Along the lower part of the foot is a verrucoid growth, which covers the dorsal surface of the toes and part of the foot, and is well raised above the surface of the skin. The growth extends around the foot but does not involve the sole. The growth is more advanced along the outside than along the inside of the foot, and extends higher up. The appearance is as if the foot was covered with old dried moss.

On the dorsal surface of the toes the growth appears as small, well-defined, but closely collected papillae, resembling a miniature forest of coarse points ; these spring from a bed of thickened tissue, well raised above the surface of the skin. The growth covers the whole of the dorsal surface of the toes, extends and overhangs or surrounds the toe nails, but does not completely envelop them. The skin between the toes is free from all growth. The growth extends slightly further on to the second and third and little toes, but does not invade the plantar surface. The big toe has some old scars, where the growths have been burnt with caustic.

The forest-like growth of warts extends towards the back of the foot for nearly 8 cm., and continues in a wavy outline across the foot. On the outside of the foot the ridge of the growth is more irregular, varying from 4 to 7 cm., and, at the back of the heel, from 3 to 4 cm. deep. It does not cover the deep sulcus at the back of the os calcis. On the inside surface of the foot the outline is still more irregular, being from 2·5 to 6 cm. wide.

The character of this growth alters on the sides and heel ; instead of appearing moss-like, the hypertrophied patches of papillae form elevated plaques, with a thick and horny or sodden epidermis. The individual points of the papillae are ill defined, and the plaques are intersected by deep cracks or fissures. On the inside of the foot the growth appears as warty, rounded, nodular masses, which project 4 to 11 mm. above the skin. Over the front of the foot are one or two small scars of a keloid character. The papillomatous growth over the toes varies from 3 to 9 mm. in thickness.

The shading of the growth is most striking. It resembles, both in appearance and colour, the old dry moss found growing on rocks which are exposed to very little moisture, the colour varying from yellow to slate grey.

On the left leg (Plate VIII, fig. 1) are two patches, measuring 1·3 by 1·7 cm. and 1·8 by 2·7 cm. They are indurated, warty growths which are beginning to ulcerate. On the lower third of the leg a large ulcer, 4·3 by 4·0 cm. is seen. The induration of the sides is extensive, and the centre of the ulcer is slightly excavated. All three ulcers are covered with a thin watery discharge, containing pus cells, granular debris, numerous staphylococcus aureus and citreus, also many bacilli of varying sizes, negative and positive to Gram.

The patient states that these three ulcers had originated as small nodular growths, resembling the one on the right leg just below the knee, and that traumatism caused these nodules to swell. Then little vesicles appeared and an ulcer resulted. He believes that the nodules appeared some three months before his visit to the hospital.

Some of the verrucoid growths over the toes were burnt with the thermo-cautery before being excised. A small portion, with aseptic precautions, was inserted under the skin of the nose of a rabbit. The incision was closed with collodion. The wound healed, and six weeks later a small vesicular eruption appeared. The vesicles burst and clear watery fluid exuded. From the middle of the cluster, minute hard nodules developed. The tissue around the base of the nodules gradually became more infiltrated. Ten days later the nodules commenced to resemble minute warts, which were evidently itchy, as the rabbit was continually scratching the spot and causing them to bleed. The papillae became encrusted with dried exudate, the patch measuring 1·3 by 2·0 cm. above the surface of the skin. The hind paws became infected from the scratching, and finally a moist warty condition was noted on the ears.

Microscopically the growth on the rabbit's nose resembles the verrucoid growth on the right foot and the ulcerated nodules on the left leg. The condition appears to be an infective keratosis. Owing to ocular troubles I have been unable to include the histological study of the tissues, and must defer the completion of this report.

Verrucoid growths are common in elephantiasis, but I have not found any reference to experimental transmission of the growths.

I do not report the condition as a case of elephantiasis, complicated by keratosis Only two of my patients have had definite enlargement of the limb. Some of the cases had warty growths on both lower limbs. I was shown a case in Pará where the growths involved the right foot and leg as far as the mid-thigh, and the left knee was covered with large patches of warty growths, some of which exhibited extensive ulceration. Dr. Stephens has allowed me to reproduce a photograph which was sent by Dr. J. H. H. Harrison, of Belize, British Honduras (Plate IX, figs 1, 2) The appearance of the growths is the same as in my cases.

Plate VII.

Front view of right foot showing verrucoid condition. Note the small nodule on the front of the leg below the knee.

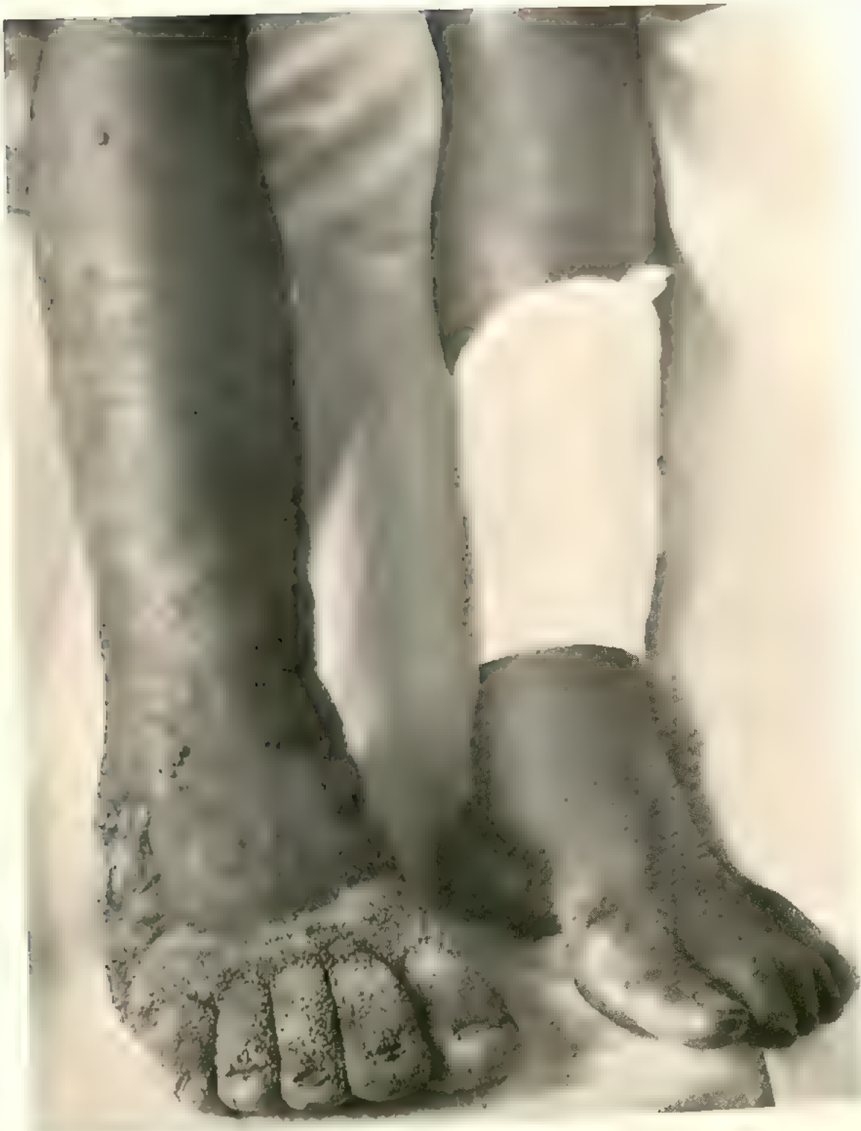

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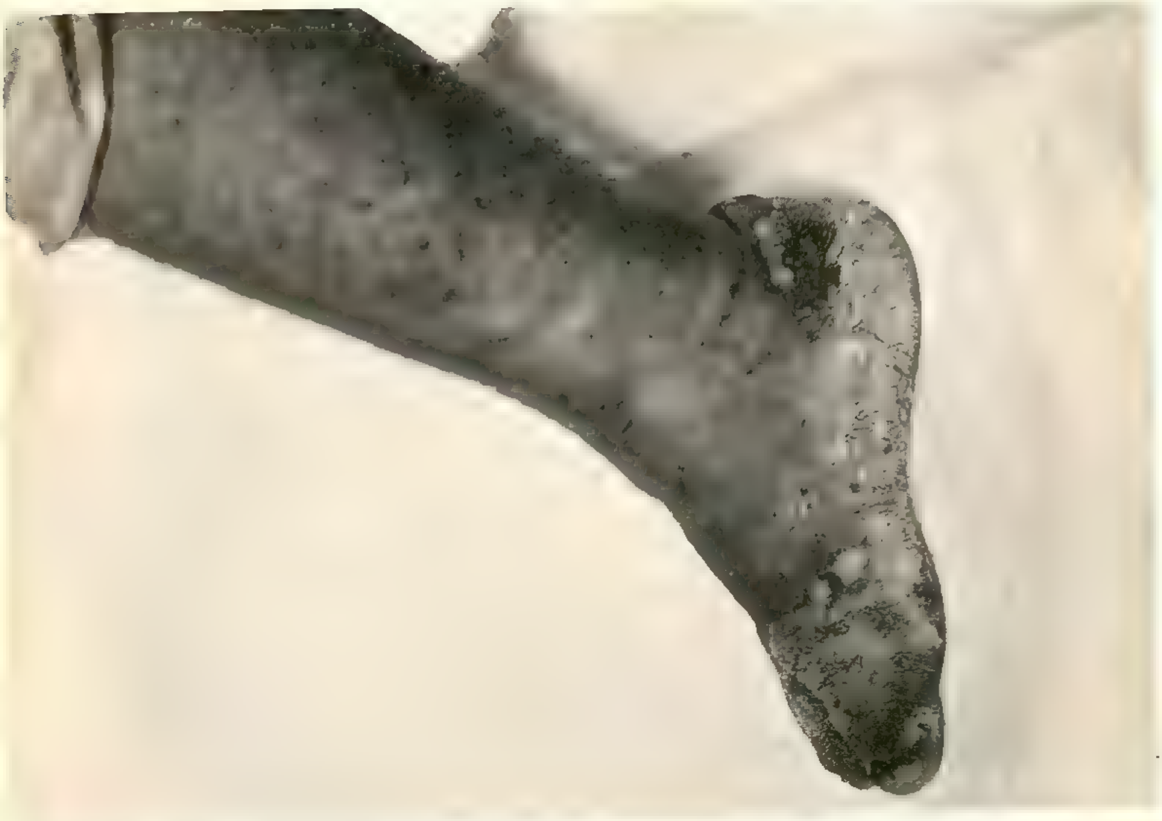
PLATE VIII.

Fig. 1. Side view of right foot. Note the ill-defined, warty, fissured masses around the heel and side of the foot. The left leg shows the ulcerated condition of two nodules.

Fig. 2. Right foot showing the thickened condition of the leg and the deep sulcus at the back of the foot.

PLATE VIII.



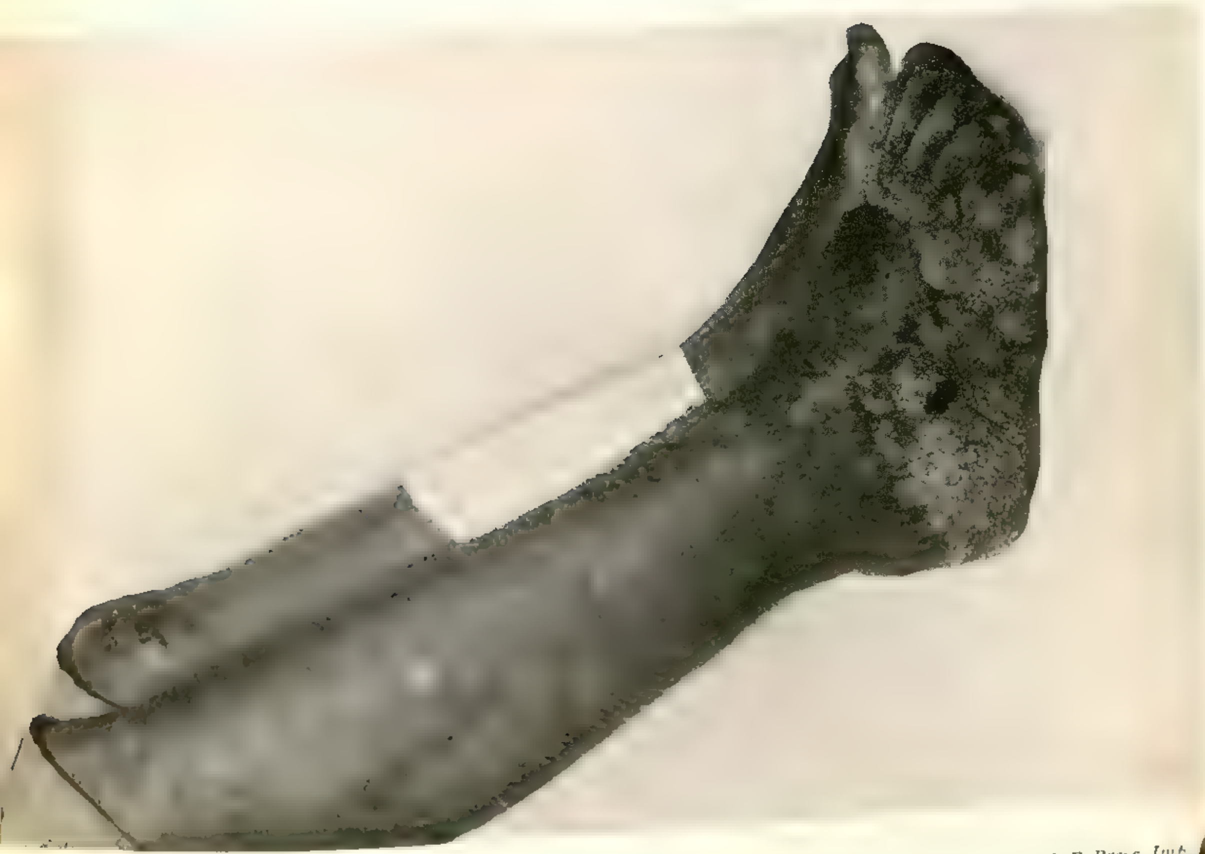


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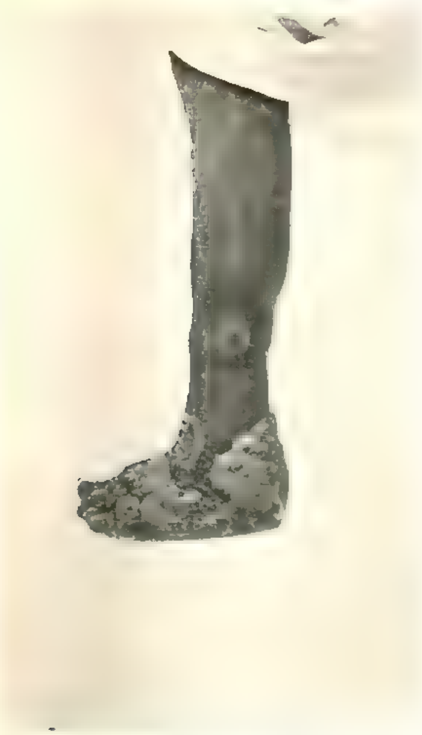
PLATE IX.

Fig. 1. Dr. J. H. H. Harrison's case. Left leg, side view, showing the same warty growths on the dorsum and sides of the foot and leg.

Fig. 2. Front view of same case.

Fig. 3. Rabbit inoculated with some of the deeper tissue of the growths. Note the nodular mass on the nose.



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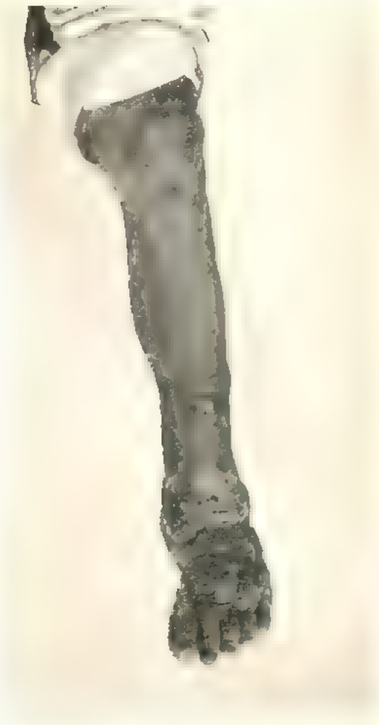

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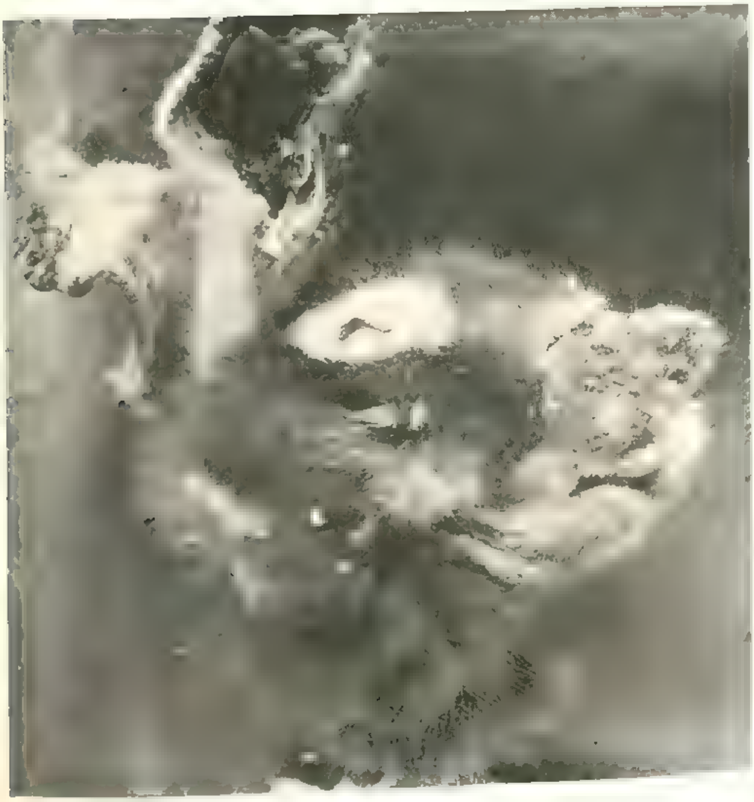

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GUARANA

BY

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(Received for publication January 11, 1910)

(Plate X)

Guarana is a Brazilian drug obtained from the seeds of a climbing plant of the natural order Sapindaceae, *Paullinia sorbilis*, Martius (*P. Cupana*, H. B. and K.). The name is derived from a tribe of Indians in the Amazon basin, and is pronounced with the accent on the last syllable, much like Panamà.

NOMENCLATURE

Paullinia,[1] Linn. Gen. ed. I, 116 (1737), Sapindaceae, Benth. and Hook, f. I, 394; Enourea, Aubl. Pl. Gui., I, 587 (1775); Semarillaria, Ruiz and Pav. Prod., iv. 52, t. 9 (1794).

Paullinia sorbilis, Mart. Reise Bras., i. 311, nomen = *P. Cupana*.

Paullinia Cupana,[2] H. B. and K., Nov. Gen. et Sp. V, 117, Venezuela.

The name Paullinia[3] is derived from C. F. Paullin, a German botanist, 1712. Shrubby tropical plants. About 125 species, mostly tropical America, one African.

Bentham and Hooker,[4] writing in 1862-1867, say of the genus: 'Species ad 80, nobis cognitae v. bene descriptae, Americam praesertim orientalem incolentes, quarum una in Africam occidentalem vagatur.'

Bentley and Trimen, Medicinal Plants, Vol. I, No. 67, give a coloured illustration of *Paullinia sorbilis*, Mart., which is reproduced (fig. I) by kind permission of Messrs. J. & A. Churchill.

HISTORY

The plant which yields guarana is abundant in the province of Amazonas, along the banks of the Tapajos, Rio Negro, as well as in

Guiana and Venezuela.[5] It has been used from time immemorial by the numerous tribes inhabiting the Amazon basin, in the form of powder made by grating the stick of guarana into water to make a refreshing drink.[6] 'Besides its medicinal properties, this substance has a reputation for affording a refreshing beverage similar in its effects to tea and coffee. It is grated into a powder, very like powdered cacao in appearance. Two spoonfuls of this powder are mixed in a tumbler of water, and this drink is regarded as a stimulant to the nerves, and, like strong tea or coffee, is said to take away the disposition to sleep.'[7]

Dorvault[8] states that guarana was first brought to Europe by Cadet de Gassicourt in 1817, and this date is confirmed by Tschirch.[9] It was not, however, until 17th September, 1822, that Mérat received a large consignment of the drug from M. Gomès. This was sent without any details as to its preparation, but mentioning that one or two drachms were rasped into a glass of water by means of a very rough bone, which was usually sold with the drug. Martius seems to have informed Mérat that the juice of the plant, roughly reduced to an extract, formed the guarana of commerce, and Mérat[10] adds that the odour of the sample suggested the presence of added cacao powder.

Hanbury[11] describes guarana as 'the basis of a favourite beverage in some parts of Brazil.' He does not seem to be aware of any special medicinal action of the drug, nor have I been able to find any further reference to it in any of his publications. Guarana is not spoken of in the standard text book of Pereira, [12] nor in an older work by Woodville.[13]

PREPARATION AND DESCRIPTION

Guarana is official in the Spanish Pharmacopoeia[14] under the heading 'Paulinia,' where it is described as a product obtained by trituration with heat of the seeds of *Paullinia sorbilis*, Martius, a Sapindaceous plant of Brazil. Roundish, ovoid, or cylindrical masses of 150 to 200 grammes weight, brittle and of brownish-black colour externally, irregular, granular and of brownish-red fracture; occasionally the remains of the seed coat are met with, or specks of added fecula are seen. Characteristic weak odour and bitter and astringent taste. Should contain at least three per cent. of caffeine

Therapeutic action astringent, tonic and analgesic. A well-known Brazilian work on Materia Medica[15] printed and published in Paris gives the following account of the drug:—'The fruits appear in capsules which, when ripe, are of a beautiful ruddy brown colour. The seeds, which are dark-coloured and almost the size of hazel nuts, are roasted, powdered and afterwards massed with some tapioca and water and are put into an oven to dry and become hard. This prepared guarana appears in the form of elliptical or cylindrical lobes of a red or ashy-grey colour with a bitter taste without appreciable astringency. It is hard, difficult to powder, but softens in water.' The Guarani Indians[16] prepare the guarana by first washing the seeds; they are then lightly heated to separate the kernel from the seed coat, complete separation being performed by beating the seeds, contained in bags, with sticks. They are then crushed upon a heated stone and made into a paste with water, to which is sometimes added cacao powder or manioc flour. This paste, moulded into cakes or cylinders, is then exposed to the sun or submitted for several weeks to a gentle heat.

Tschirch[17] states that Guarana in sticks comes over in chests of 65 kilos.

The cylindrical form is that in which the drug is usually seen, and Fig. 3 taken from portions of two such pieces (No. 463) in the Museum of Materia Medica of the University of Liverpool, gives a fair idea of this form. Fig. 2 shows a specimen (No. 288) of the seeds in the same collection.

In addition to the forms mentioned above, guarana is occasionally seen in this country in other shapes, such as those of fish, animals, models of the plant yielding the drug, and of other plants. This is well seen in Fig. 4, taken from a specimen (No. 806) presented to the above Museum by Dr. H. Wolferstan Thomas. This is a model of the fruit, of the plant yielding the seed, from which the beverage cocoa is prepared, *Theobroma Cacao*, L. Dr. Thomas tells me that he has frequently seen specimens of the drug in these curious forms, and that it is worth about three shillings a kilo. In 1871 it was worth about eight pence a pound, and could be bought on the Rio Negro at as low a rate as a penny a pound. The present market price is about twelve shillings a pound.

The paste used in making these models is finer than that used for the other forms in which the drug is sold, and the models are neatly executed.

Holmes[18], referring to a specimen in the Museum of the Pharmaceutical Society of Great Britain, says:—'This specimen, presented by Dr. Wilks, is accompanied by a letter from Mr. G. H. Brandt stating that it has been carved by the Indians into the shape of a fish called the Piraruccu (*Arapaima gigas*, Cuv.), the tongue of which is used for grating the Guarana. The tongue is also used by them as a file for grating several of their hard odoriferous roots. Amongst these is the root of the Piperiocca, which, when reduced to powder, they use for washing and perfuming their bodies. The so-called tongue consists of a long bony plate, nearly six inches long by one and a quarter broad, densely covered with bony papillae, about a quarter of an inch high and half a line broad. See *Cuvier and Valenciennes*, Hist. Poiss., XIX, pp. 441-461, with fig.; *Agassiz and Spix*, Pisc. Braz., p. 31.'

It is probable that this bony plate is a similar one to that mentioned by Gomès.

Tschirch[19] speaks thus of the curious forms in which drugs are sometimes seen: 'At times the finished products are further worked up. From Caoutchouc and Guarana paste, were formerly made all sorts of curious figures, but now Guarana is usually seen in sticks.' He illustrates this in the case of Caoutchouc.

Microscopic Characters

These are well described by Planchon and Collin, and consist chiefly of swollen starch grains, needle-shaped crystals, small polygonal cells, and characteristic sclerenchymatous cells.

Chemical Constituents

'The active chemical principle is an alkaloid first discovered by Dr. Theodore von Martius, and called by him guaranine, but since shown by Dr. Stenhouse[20] to be identical with theine. Guarana shows more than double as much of this alkaloid as good black tea, and five times as much as coffee, the proportions being 5·07 per cent. in guarana, 2·13 per cent. in tea, and 0·80 per cent. in coffee. The same alkaloid is found to the amount of 1·25 per cent. in Matè, or Paraguay tea, the produce of several species of *Ilex*.

It is rather a singular coincidence that the same alkaloid should prevail in all the principal substances employed in a similar manner as beverages in different parts of the world—in the tea of China and India, the coffee of Arabia, the Cacao of Central America, the Matè of South America, and the guarana of Brazil. M. Fournier[20] has found in the last-named substance, besides tannate of caffein, the following principles:—Gum, starch, an acrid green fixed oil, a concrete volatile oil scarcely soluble in water, a peculiar principle not precisely determined, and tannic acid.'

Tannic Acid was found in guarana by F. V. Greene,[21] and he describes the reactions of this as 'strikingly dissimilar from those of tannic acids generally.' Thoms,[22] in 1894, found in guarana 8·63 per cent of moisture. Later work by E. Kirmsse[23] showed the presence of tannin. From the crude tannin this worker obtained crystals of a catechin the properties and reactions of which were found to be identical with that of Pegu catechu. The author confirms Thoms's statement that the amount of caffeine in guarana has been over estimated; three samples were found to contain 2·68, 2·97 and 3·10 per cent. respectively. Cocoa, which has been stated to be an adulterant of lower grades of guarana, was not detected in any of the samples.

Oesterlen[24] notes also the presence of a resin, and says that the tannin of guarana appears to act like that of Monesia and Rhatany.

A large number of authorities[25] have reported the presence of *Caffeine*, which is the most important constituent of guarana, and identical with guaranine and theine. In 1853 Oesterlen[26] refers to a special indifferent crystalline substance guaranin. Hanbury[27] speaks of caffeine as a constituent of guarana. There is some variation in the statements of authorities as to the percentage of caffeine present, thus we have the following data:—

In 1886, 4·5 per cent. Bochefontaine and Gusset.[28]
3·72 to 5 or 6 per cent. Kremel, Feemster, Squibb and Flückiger.[29]
1888, 3·12 to 3·80 per cent. A. Kremel.[30]
1894, 2·6 per cent. Thoms.[31]
1897, 4·32 to 4·68 per cent. C. H. La Wall.[32]
2·5 to 5 per cent. Humphrey[34] and British Pharmaceutical Codex.[35]

Ash

The yield of ash is stated to be :—

In 1886, 1·36 per cent. H. Warnecke.[36]
1888, 1·30 to 2·00 per cent. Kremel.[37]
1894, 1·68 per cent. Thoms.[38]

I have been unable to find any further reference to the peculiar principle mentioned above by Fournier.

Medicinal Properties

Oesterlen,[39] in 1853, states that the powdered seeds were given for diarrhoea and similar complaints by Ritchie in the dose of one drachm per diem (Ed. Journ., May, 1852). Dorvault[40] says that guarana was introduced as a tonic and astringent, which is the use made of it in Brazil, and that it has been advocated as a remedy for neuralgia and migraine. A dose of 4 to 8 grammes was given, and there was a stock of it in the French pharmacies in 1867, when it was made into two syrups, tincture, pills, ointment and a chocolate Chernoviz[41] mentions that guarana is prescribed as medicine in various affections, chiefly in diarrhoea and dysenteries, in doses of two drachms in eight ounces of water. Speaking of guarana in 1872, a writer in the Year Book of Pharmacy[42] says :—'This drug is now attracting attention as a remedy for sick headache. It has been long known, but has never come into general use.' Mr. Harold Wyatt, who has had some years' training in French pharmacy, tells me that the drug is more used in France than in England, and my own experience of the two countries proves the same, as since 1884 I have seen guarana prescribed but three times, in cachets, and two of these occasions were whilst I was in France, but it appears that the elixir is sold to a considerable extent in Liverpool. Guarana is official in the pharmacopoeias of Austria, Belgium, Dutch Supplement, France, Hungary, Italy, Mexico, Portugal, Spain and U.S.A.,[43] and our own British Pharmaceutical Codex[44] has a monograph upon it, in which it says :—'Guarana is used for the same purposes as caffeine; it has a reputation in sick headache, and is sometimes used as an astringent in diarrhoea and dysentery. The drug is used in powder form, being given in a cachet or mixed with water to form a draught. Elixir of guarana is a pleasant liquid form of the drug. Tincture of guarana is suitable for use in mixture form. Dose : Half to one gramme.'

The Addendum to the Austrian Pharmacopoeia VIII[45] has a Pulvis Guaranae Compositus of the following composition: Pulv. Guaranae 5, Sodii Salicyl. 3, Quininae Sulph. 2, in capsules each to contain a gramme.

The United States Pharmacopoeia[46] has a standardised preparation, Fluidextractum Guaranae, containing 3·5 per cent. alkaloid made by percolation with diluted alcohol. The average dose is half a drachm.

Allied Plants

The natural order Sapindaceae, and particularly its sub-order Sapindeae, includes many interesting plants having medicinal action.

Em. de Maout and J. Decaisne[47] state that *Serjania* and *Paullinia*, American genera, are poisonous; the Brazilians use their juice to stupefy fish.

Paullinia cururu. The juice of this plant is used by the natives of Guiana to poison their arrows.

P. pinnata, L. A poison is prepared from the root and seeds, and the expressed juice of its leaves furnishes the Brazilian Indians with a powerful vulnerary.'[47], Créteur[48] says that *P. pinnata*, L., is a powerful climber, the seeds of which are used as a stupefacient in the Antilles and Brazil, and act upon fish in the same way as the Coque du Levant (*Cocculus indicus*). The leaves, called 'cururu ape,' are vulnerary. *P. pinnata*, L., is also one of the drugs called 'Timbo,' which is the name given in Brazil to several plants, such as *Serjania cuspidata*, *S. lethalis*, of the order Sapindaceae, and *Tephrosia toxicaria*, and *Physalis heterophylla* of the order *Leguminosae*, all of which are used for the purpose of stupefying fish. A decoction of the root is preferred, as affording the more powerful poison.

Planchon and Collin[49] state that the bark usually used for fish poisoning in Brazil is that of *Serjania curassavica*, Radk. (*P. senegalensis*, Jus., *P. africana*, Don.), and give Martius as authority for the statement. This plant grows in Brazil, Mexico, Guiana, the Antilles and West Africa. *P. pinnata* is also reported as growing in West Africa.[50] *P. costata*, Schl., is used in Mexico to kill fish.[51] *P. mexicana*, L., or quarhmetcatl of the Mexicans, possesses the virtues of sarsaparilla in the treatment of rheumatism and syphilis.[52]

Greshoff[53] gives a very complete list of Indo-Malay poisons, and amongst them are *Serjania*, *Cupania* and *Paullinia*.

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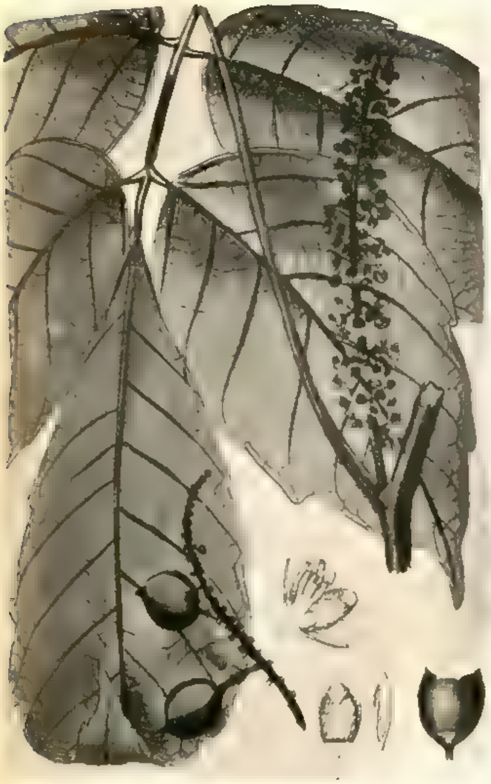

Fig. 1.
Paullinia sorbilis, Martius.

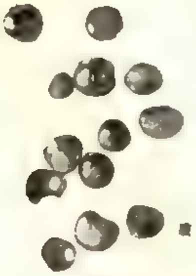

Fig. 2.
Seeds of *Paullinia Cupana*, H. B. & K.

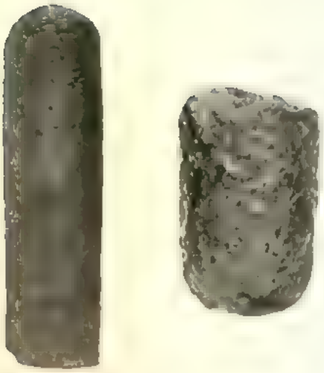

Fig. 3.
Guarana in sticks.

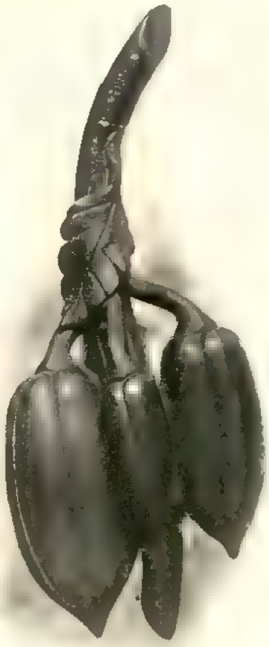

Fig. 4.
Guarana paste in form of fruit of *Theobroma Cacao*, L.

P. P. Press. Imp.

SOME OF THE CHEMICAL CONSTITUENTS OF GUARANA

BY

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The guarana used for this investigation was kindly given to me by Dr. Wolferstan Thomas, who informed me that the drug is used as a remedy against diarrhoea in Brazil. The work was carried out by me while still on the staff of the Liverpool School of Tropical Medicine, in the Runcorn Research Laboratories.

As far as my present results go, the chief and probably the effective constituent of the particular guarana[1] examined by me is the alkaloid $C_{40}H_{47}O_{21}N_4$, for which the name *β-guarinine* is suggested. The *β-guarinine* differs from Martius's[2] guarinine, isolated by him from *Paullinia sorbilis*, in that I was unable to identify it either with theine or with theobromine, whereas guarinine is, according to Berthemot and Dechatelas,[3] simply theine This is of interest, as the alkaloids both of *Paullinia sorbilis* (v. *cupana*) and *P. cambess* are either theine, theobromine, or a mixture of both alkaloids. However, it ought to be mentioned that neither of those two species of *Paullinia* are used for diarrhoea, according to Dekker,[4] who states, *only* with reference to *Paullinia trigonia*, Vellos: 'de zwak gerooste zaden teger diarrhoe.' This probably accounts for the fact that I was unable to find in the guarana I examined, an alkaloid of a known purine type.

β-guarinine

The finely powdered material was extracted with alcohol containing 1 per cent. of hydrochloric acid, at a temperature of

55 to 60° C. on the water-bath. After cooling, the calculated amo of ammonia was added and the product—a greyish powder—collected and re-dissolved in boiling alcohol. The alcoholic solution was then boiled with animal charcoal for ten to fifteen minutes, filtered and evaporated to about one third of its volume, when on standing s needles crystallised out. These needles melted at 217° to 219°, but at a temperature of 167° to 169° a slight decomposition was noticed. The product was dried for two hours at 110°, when a loss of water of crystallisation corresponding to $2\frac{1}{2}$ molecules was observed.

The following analyses were carried out:—

I. 0·2151 gr. gave 0·3744 gr. CO_2 and 0·1065 gr. H_2O.
II. 0·2001 gr. gave 0·3474 gr. CO_2 and 0·0979 gr. H_2O.
III. 0·2337 gr. gave 0·4057 gr. CO_2 and 0·1109 gr. H_2O.
IV. 0·2211 gr. gave 18·8 c.c. N_2 (moist) at 27° C. and 741 mm.
V. 0·2473 gr. gave 20·6 c.c. N_2 (moist) at 27° C. and 741 mm.

Found C = 47·47%, 47·35%, 47·35%.
H = 5·50%, 5·44%, 5·27%.
N = 9·24%, 9·00%.

$C_{40}H_{47}O_{21}N_4$ (β-Guarinine) requires: C = 47·00%, H = 4·90%, N = 9·14%.

$C_8H_{10}O_2N_4$ (Theine) requires: C = 49·48%, H = 5·16%, N = 28·86%.

$C_7H_8O_2N_4$ (Theobromine) requires: C = 46·66%, H = 4·44%, N = 31·11%.

On boiling with acetic anhydride, β-guarinine forms an acetyl derivative—probably the monoacetyl (?)—which crystallises from alcohol in small needles and melts at 192° to 196°.

When treated with methyl iodide, β-guarinine forms the corresponding $C_{40}H_{47}O_{21}N_4CH_3I$, which is easily transformed into the free base $C_{40}H_{47}O_{21}N_4CH_3OH$ on treatment with freshly prepared Ag_2O. The base crystallises from chloroform in long needles, which melt at 167° to 168°.

Like other alkaloids, β-guarinine forms, when brominated in chloroform, a bromine derivative, probably $C_{40}H_{46}Br\ O_{21}N_4$, which crystallises from acetic acid and alcohol, and melts at 236° to 237°.

From the above analytical data it is obvious, especially from the nitrogen-figures, that β-guarinine is neither theine nor theobromin

Similar conclusions must be drawn from a comparison of the different melting-points of β guarinine and its derivatives, with those of theine or theobromine and their derivatives, as can be seen from the following table:—

	β-Guarinine	Theine	Theobromine
Free base	217–219°	234–235°	329–330°
Acetyl derivative....................	192–196°	Not known	Not known
Methyl..................................	167–168°	137–138°	119–121°
Bromine	236–237°	205–209°	Not known

A great deal of attention has been paid by other workers to the tannic acid,[5] the so-called *guarana-tannic* acid, found in guarana, but I have been unable to confirm their results, viz., that guarana-tannic acid is identical with catechin. On the other hand, my results point more or less to the fact that this particular acid is identical with *Chlorogenic acid*, isolated by Gorter[6] from Liberian coffee-beans. Gorter has recently proved that coffee-tannic acid does not exist, and has shown this acid to be a mixture of chlorogenic and coffalic acids. He[7] has also shown the so-called *Heliant-tannic acid* from *Helianthus annuus* to be chlorogenic acid.

A quantitative estimation of the tannic acid in the guarana carried out by Korner's and Nierenstein's[8] caseine method gave 4·3 per cent, an amount which seemed to be sufficient for an attempt at isolating it. The acid was accordingly isolated by the acetone-method[9] and purified by formation of its lead salt. It represented an amorphous powder of no definite melting point. It was further purified by dissolving the product in ethyl acetate, the solution, after filtering, was evaporated to dryness and re-dissolved in boiling water, from which solution it slowly separated out in small colourless needles, which melted at 199° to 201°.

For the analysis the product was dried for two hours at 110°.

0·1886 gr. gave 0·3664 gr. CO_2 and 0·0877 gr. H_2O.

Found, C = 52·98%, H = 5·17.

$C_{32}H_{38}O_{19}$ (Chlorogenic acid) requires: C = 52·90%, H = 5·22%.

$C_{15}H_{14}O_6$ (Catechin) requires: C = 62·06%, H = 4·83%.

These results, which indicate that guarana tannic acid may be identical with chlorogenic acid, were *not* confirmed either by formation of the acetyl derivative or the aniline salt; both preparations had quite different melting points from those given by Gorter. This leads us to the view that the tannic acid from guarana is not identical with chlorogenic acid, but that it is as an independent guarana-tannic acid; and in no case can this acid be considered to be catechin, as can be seen from the following table of melting points:—

		Acetyl derivative	Aniline salt
Guarana tannic acid	M.P. 199–201°	M.P. 147–149°	M.P. 203–205°
Chlorogenic acid	M.P. 206–207°	M.P. 180–181°	M.P. 174°
Catechin	M.P. 175–177°	M.P. 124–125°	Not known
Perkin's & Yoshitaka's higher-melting Catechin	M.P. 235–237°	Not known	Not known
Acacia Catechin	M.P. 204–205°	M.P. 158–160°	Not known

Further investigations of both the β-guarinine and the guarana-tannic acid are wanted, before a definite opinion with regard to the constitution of these two compounds can be formed; *however, in no case can the opinion be maintained that the chief constituents of this plant are theine and catechin.*

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YELLOW FEVER

BY

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(*A Lecture delivered to the students of the Liverpool School of Tropical Medicine.*)

We have now to deal with the prognosis and treatment of one of the most serious of all diseases to be met with in the tropics and sub-tropics, and, unfortunately, still a menace to the lives of many of the young foreigners who come to make their livelihood in an endemic district, or to the residents of a region stricken by an epidemic of the disease. We who work in endemic or epidemic regions, have still to urge and demand that the endemic areas shall be abolished, and that the places where the disease may appear perhaps at any time or during certain months in the year shall take the necessary steps to place the district in such a state that it would be impossible for an epidemic to occur, even if some cases of yellow fever were introduced into the area. The way to attain that end is Tropical Sanitation. Tropical sanitation implies a general cleaning of a town, a campaign against mosquitos and the training of the inhabitants to recognise that certain practices are dangerous to the city and the residents. If an anti-mosquito campaign is instituted it means the abolishing of breeding spots, the screening of all receptacles used for the storing of water, and the general cleaning of the city, the freeing of back-yards from the accumulations of rubbish and the compelling of the inhabitants to keep their premises clean. Thus do we fight yellow fever by giving the mosquito, *Stegomyia calopus,* as little opportunity to breed as possible. Ross, Gorgas and others have shown we are able to free a city of yellow fever only when we have so reduced the numbers of the mosquito that, even should a case of the disease be introduced into the community, the Stegomyia will be so few in number that there will be very little chance of their biting the patient In former times the inhabitants of epidemic areas went in dread of an outbreak. It meant, in the Southern States of the United States, a 'shot-gun quarantine: as a result the residents and their city were shunned by all and a general stagnation of trade ensued Such methods however, are of the past. New Orleans has demonstrated what a scientific combat with Stegomyia can do, and she has been added to the list of successes which have always occurred where the Havana anti-yellow fever measures have been instituted. Havana and Rio de Janeiro, both endemic centres, are free of the disease, and Panama has shown how an immense engineering scheme can be executed without repeating the old tale of wholesale loss of life from yellow fever.

Despite the success of these measures in the places I have mentioned, other countries and towns have not followed suit, and in them yellow fever continues to be a scourge, and they constitute a danger-zone to all places within the limits of the Stegomyia

Yellow fever is a disease which allows of little delay. It runs its course in a few days, and you must be prepared to offer battle. Fortunately many cases are of a mild type, and unless something exceptionally wrong is done the case will recover. Such cases are good for your reputation, and as yellow fever exacts a heavy toll, and as it will probably be your misfortune to lose many cases, you will need them. No one who treats many cases of the disease can avoid losing a certain percentage of his patients. There is no panacea for yellow fever as there is for malaria, but a good physician can, by his management of the case, markedly reduce the mortality.

Eliminative and supporting measures, with the reduction of the congestion, should be our aim. Briefly, we can say that treatment depends on nursing, abstinence from all food, rest in bed, and hydrotherapy in the form of alkaline waters to act as mild diuretics. Water is a very powerful remedy in this disease, and should you ever have to treat many cases of yellow fever, you will be surprised at the number of patients that recover without any other treatment than alkaline waters and good nursing.

Various types of yellow fever are met with. In adults, the extremely mild forms resemble influenza or a disturbance of the digestive system: *Embaras gastrique fébrile.* In young children the types are milder. These forms are rarely diagnosed, and the patients recover after a few days. The danger is that the cases remain unscreened and serve to infect Stegomyia. The types which require treatment are numerous, and I believe it will be as well to describe an average case, and to call your attention to the pathological lesions met with in this disease; and if you will only bear in mind what macroscopic and microscopic changes are occurring in some of the organs of the body, you will be able to appreciate better the reason for the occurrence of black vomit, anuria, etc., and the multiplication of symptoms and complications seen so frequently in this disease.

You will probably be called some morning to see a patient who will give a history that he had been at work the day before and had gone to bed feeling well. Some hours later he woke up feeling uneasy, with a certain amount of frontal headache and nausea. He possibly shivered or had an actual rigor. He began to notice that his back and lower limbs ached, and gradually his symptoms increased.

On your arrival you find your patient tossing about in bed because of his pains. He will have vomited once or twice; his headache is very severe and increasing; his eyes are bright and shining and sensitive to light and, as he lies in bed, you note the general congested appearance of his head and shoulders. The mucous membranes of the lips are bright red, the conjunctivae show slight congestion, the tongue is reddened and slightly coated, the papillae appear as bright red points. The temperature a few hours after the onset will be 100 to 101° F. (37·8 to 38·4° C.); the pulse 100 to 110, hard and bounding; the respirations slightly quickened. Your patient may have vomited the remains of the last meal or some bile-stained mucus and complain of the acidity of the vomited matter. The skin is usually moist; the urine is generally scanty and high coloured. Such are the usual symptoms in a case of eight to ten hours' duration, symptoms which are common to all acute fevers.

In a few hours, i.e., eighteen hours after the onset, the appearance of the case is much worse. The congestion has increased, and the face, ears, neck and upper part of the chest are tinged a dull crimson, more or less of a boiled lobster shade. The mucous membranes are congested and tinged. The conjunctival injection has increased, the eyelids through the engorgement of the capillaries are somewhat swollen and everted, and the space between the upper and lower lids is narrowed, causing the patient to present a ferrety-eyed appearance. The headache is splitting and is usually occipital and frontal. The least movement increases the pain, and the patient may feel as if his brain was hammering through the skull-cap. The tongue is generally coated with a light dirty yellow fur; the tip and sides are red and clean, the scarlet papillae are very distinct. On examining the gums small capillary injections are noted about the teeth along the 'lead line.' The mucous membrane of the inner sides of the cheeks is injected, and on examining the floor of the mouth around the base of the tongue, very minute bright red points will be seen. These are especially noticeable in yellow fever, and I have rarely found them so marked in any other febrile disease. Even in mild cases they are easily observed. Always examine your cases in strong, bright daylight, as it is impossible to ascertain the colour by artificial light.

The radiating pains in the loins (le coup de barre) and legs are intense. The limbs feel leaden. No comfortable position can be found, and the position in bed is being constantly altered. Very often the patient is driven to cry out. The soles of his feet throb. I have had patients insist that they felt the toe nails lifting and throbbing.

Thirst is a prominent feature and practically unquenchable. The nausea has lessened and vomiting ceased, unless the case is an alcoholic or one with a history of gastritis. When vomiting continues it may prove to be most troublesome and dangerous. Probably the patient has not slept at all, and although feeling so tired and weary sleep will not come. The mind is active, the patient notices everything, and is very suspicious of his attendants and his surroundings. Albumin is rarely found so early. The temperature has now risen to 102° or 103° F. (38·9° to 39·4° C.). The pulse is 110 to 120, still bounding and hard, but regular. The respirations are about 25. About the twenty-fourth hour the pulse may not show an increase, although the temperature may still be rising. Faget's sign, the slowing of the pulse with a steady or even rising temperature, can often be observed.

At the thirty-sixth hour the fever has probably reached a maximum of 101·5° to 103° F. (38·6° to 39·4° C.) in mild cases, 103° to 104° F. (39·4° to 40° C.) in severe cases, and 105° to 106° F. (40·5° to

41·1° C) in extremely severe ones. In very virulent and practically fatal cases, the temperature may rise even higher. The pulse may have diminished ten or more beats.

Although the vomiting has ceased there may be more tenderness over the epigastrium and sensations of burning or pain are felt. The thirst still continues The skin is less moist. The cephalalgia and pains in the back and limbs are acute. The urine is probably free from albumin and still scanty in amount.

By the end of the second day the pulse begins to fall to 100, 90 or 80, and the temperature has remitted one or two degrees. The tongue is still coated, the injection of the mucous membranes persists but is lessened The congested appearance of the head is less marked, the eyes are less suffused, the headache and the pains in the loins and limbs are ameliorated. The patient feels better. The urine probably contains a little albumin, which gradually increases in amount.

During the next twenty four hours the temperature commences to fall, dropping as much as one to two degrees, the pulse decreases but at the same time it becomes irregular and without tone. The albumin increases slightly; jaundice is usually apparent. The general symptoms of the patient improve, and, if it is a mild type of the disease, the temperature and pulse will gradually fall to normal or subnormal by the fifth or sixth day, all the symptoms decreasing by degrees. In a more severe type defervescence is not so rapid; there may be epistaxis, some oozing from the gums, and the albumin in the urine may have increased from 2·0 to 3·0 grammes per litre. These symptoms only persist for a short time. The temperature and pulse usually remain subnormal for six to fourteen days. Bradycardia is common, and a pulse of 40 or 35 is not unusual.

If you recollect the microscopical appearance of the organs of a fourth day case, you will remember that up to this time the organs have only shown congestion and slight cloudy swelling, a commencing periglomerulitis, swelling and desquamation of the epithelium of the tubules with congestion of the small vessels in the cortex of the kidney. The liver exhibits greater changes, and, even on the third day, the hepatic cells will show vacuoles in the protoplasm, and there will be an infiltration of small round cells about the portal spaces which tends to compress the lumen of the ducts and vessels. The stomach will only show congestion.

Should the patient's symptoms have not improved and the disease progress, the lesions rapidly increase. The kidneys will show all the characteristics of an acute parenchymatous nephritis, and small droplets of fat are seen. The epithelium of the convoluted tubules is especially degenerated; in many places hyaline casts are filling the lumen of the tubules. In the liver, the changes are still more evident, the fatty degeneration is well advanced, the cells show large vacuoles, and their protoplasm is granular, the hepatic lobules are deformed and the contour distorted. Around the central vein the cells are most irregularly distributed. The infiltration of granulation cells about the vessels in the connective tissue of the portal spaces has increased, and many of the vessels and ducts are obliterated. The stomach shows little points of ecchymoses, especially about the cardiac and pyloric ends; all the vessels are engorged with blood.

Remember these advanced conditions and that there is a general steatosis of all organs about the seventh day, and you will find it easy to understand the symptoms typical of all acute cases which so often result in death. There is a passive congestion due to the liver, caused by the accumulation of round cells about the vessels in the connective tissue of the portal spaces. These cells tend to compress the vessels, and the congestion is aided by the enlarged degenerated hepatic cells which compress the hepatic capillaries. Through the obstruction of the portal circulation, the blood is dammed back in the mesentery and intestine, especially about the pylorus and duodenum, where, as Carroll* points out, the vessels—the pyloric and duodenal veins—are short and with very few anastomoses. By the end of the fourth day the endothelium of the blood vessels is beginning to show signs of fatty degeneration. Under such circumstances it is no wonder that the small congested capillaries will rupture and cause black vomit. The capillaries in the stomach distend and burst, and a small ooze of blood will gradually mix with the acid secretions of the stomach. The result is the 'coffee grounds' or 'fly-speck' vomitus. As more of the capillaries rupture and ooze, the vomitus changes to a uniform blackish-grey or brown tint. It is only when a comparatively large vessel ruptures and there occurs an immediate attack of vomiting that we see bright red blood.

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* Carroll, James. The treatment of Yellow Fever. Jour. Am. Med. Assoc. July 19, 1902.

In the more severe cases the temperature will recede a little and the patient feels better; but this is only for a short time, seven to twenty hours. During the quiescent period the outcome looks rosy to the uninitiated. The patient feels himself again, his headache and pains have left him. He commences to feel hungry and demands food, and talks of being out of bed in a few days. It is hard for the onlooker to believe the improvement is only temporary and that in a few hours haemorrhages and black vomit will appear. Unfortunately such an occurrence is too frequently the case. In a few hours the appearance of the patient has altered. He is vaguely uneasy and restless The congestion of the mucous membranes is livid The epigastric tenderness which had lessened, reappears and rapidly becomes worse The liver is tender, and pressure over the epigastrium is exceedingly painful. The albumin increases in amount and the quantity of urine may decrease. The temperature will begin to rise. The pulse rate will increase but not in proportion to the temperature. Tiny coffee-ground specks may appear in the vomitus; these increase in amount; they may coalesce and become streaky (it is rare to find fresh blood coagula at this stage), when suddenly, with little warning to the attendants, black or brownish-grey vomit will occur Should the kidneys withstand the strain and continue to secrete urine, the patient can recover even if he has had several bad attacks of black vomit. If the general condition continues to become more serious, the icteric tint grows more and more pronounced. The symptoms become intensified, the black vomit may increase in frequency and amount. The patient becomes steadily worse. His mind is no longer clear, he rambles or may become delirious The tongue is dry and coated, the lips are cracked, the mucous membrane is livid. Haemorrhages from other mucous membranes occur. There may be bleeding from the lips, gums, nose and ears The epigastric tenderness is intense, pressure will elicit a cry from even a delirious patient. The prick of a hypodermic needle will often cause a small haemorrhage; pressure on the muscles may cause subcutaneous ecchymoses, and the condition may become so intense as to resemble a haemophilic state. Intestinal haemorrhages may occur The bowels will move repeatedly, and the stools will become more and more tarry and offensive. The kidneys* begin to manifest signs of the strain, the albumin is increasing rapidly, and the amount of urine is decreasing. All the symptoms point to a collapse of the renal system, but even yet the case is not hopeless provided that the kidneys are able to perform their functions. Should anuria occur and continue for more than a few hours the prognosis is of the very gravest. All this time the temperature may continue elevated, but it is generally falling and may become normal or subnormal The pulse is running; very often it is irregular, 80 to 110. The mucous membranes are extremely pale and show the effects of the exsanguination. As the state of the patient progresses towards a fatal termination, the vomiting and movements of the bowels become intensified, or convulsions and coma intervene, and death occurs within a few hours, although we have seen the anuric state last for as long as fifty-six hours With some patients the mind may be clear until the last few minutes. This is especially the case where collapse has occurred and the temperature has fallen very much below normal.

Yellow fever is always an impressive and terrible disease. It is striking because it attacks those who are robust and in the prime of manhood. The suddenness of the onset and the rapidity with which death follows must necessarily impress the relatives and acquaintances of the victim Fortunately for the sufferer, the friends and attendants, the battle is short, sharp, and decisive. Those who have passed through the ordeal generally come through unscathed. Very few permanent ill effects can be recorded.

PROGNOSIS

In the mild forms of yellow fever, recovery occurs in almost every case, provided that the patient is not already suffering from some organic disease, especially of the kidneys, but even in these cases we have seen recovery follow.

In the more severe forms of the disease, where there is a certain amount of gastric disturbance and moderate albuminuria, with care and efficient nursing the prognosis can be regarded as excellent.

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* Carroll pointed out that in the advanced stages of the disease but little urea can be formed; so that the end products of metabolism, carbonate, lactate and perhaps carbonate of ammonium are retained in the circulation and act as additional poisons. Thus anuria and ammonaemia are produced, and the delirium and coma, with or without convulsions, is due to ammonaemia and not to uraemia.

Patients exhibiting a high degree or steady increase of albuminuria and a lessening in the amount of urine voided in the twenty four hours are bound to have a severe struggle. So long as there is a fair quantity of urine passed, the amount of albumin per litre may be 2·0 to 4·0 grammes. A grave prognosis is always given when the kidneys show signs of failure and the albumin is increasing. Albumin appearing in the first twenty-four hours is an extremely serious sign. The later the albumin appears in the urine, the better the prognosis. Anuria, if long continued, is invariably fatal.

Marked jaundice on the second day indicates severe hepatic lesions, and merits a grave prognosis. The longer the appearance is delayed the more favourable the prognosis. Severe jaundice is generally associated with delirium ; it is usually the forerunner of haemorrhages, and if there is coincident failure of the urine or severe albuminuria the outlook is most gloomy.

Haemorrhages. Epistaxis is a not unfavourable sign, and the patients appear markedly relieved after they have lost some fifty or more grammes of blood. It is quite common to meet with it as one of the prodromal symptoms. Severe and long continued epistaxis is to be feared.

Bleeding from the gums is not unusual and is not dangerous, provided it only appears about the fourth to fifth day and is not excessive. An early appearance on the second or third day and copious haemorrhages indicate a severe attack, and in all probability other haemorrhagic complications will occur. Copious haemorrhages from the inside of the cheeks and the tongue are prone to occur through the patient accidentally biting the tip or sides of the latter or the mucous membrane of the cheeks.

Gastric haemorrhages are always an indication of a severe type of the fever. Black vomit occurring within the first two days of the disease is of the very gravest import. Though it is always a bad symptom and demands a guarded prognosis, patients whose kidneys are acting well can develop black vomit on the fourth or fifth day and recover. The longer black vomit is delayed the better the prognosis. Marked failure of the renal system associated with this complication is almost invariably fatal. Black vomit appearing in cases whose gums are already bleeding generally indicates a fatal termination.

When black vomit appears abundantly and frequently, the prognosis is always grave.

Other haemorrhagic conditions, such as the purpuric patches on the body, ears, etc., extravasations of blood into the conjunctivae, a general haemophilic condition when every orifice bleeds, are only seen in the malignant and fatal types of yellow fever. In women it is not uncommon for menstruation to appear before the customary time or to reappear in fatal cases. Haemorrhage may occur from the uterus, vagina or urinary bladder.

Delirium must be regarded as a grave symptom, and if occurring during the first couple of days is almost invariably fatal. Occurring after the fifth day the prognosis is favourable. One of the great dangers to be feared from this symptom is that the patient in the violence of his delirium induces other complications and cannot be properly nursed. Rambling and incoherence are common symptoms of all severe cases. A moist skin in the early stages of the disease is of good omen. The cold clammy skin encountered in collapse and afebrile forms is to be dreaded.

Temperature. The degree of fever is of value if it is taken into consideration with the other important symptoms. A high temperature associated with copious urination and scanty albumin is favourable. A moderate fever of 103° to 104° F. (39·4° to 40° C.) with an early and rapid fall is a good sign. Bad prognoses are when the temperature remains up for several days, is at a moderate level for the first thirty-six hours, and then rises, or if a temperature of 105° F (40·5° C.) and over occurs in the first twenty-four hours. Rapid defervescence on the second or third day to subnormal is to be feared if the pulse exhibits a high degree of bradycardia. A subnormal temperature associated with haemorrhage is extremely unfavourable.

A guarded prognosis should be given in alcoholics; fast livers; overworked persons; patients who persist in disobeying the nurse and doctor; patients who have delayed acknowledging they were ill, ambulatory cases or patients whose constitutions are already weakened by disease; pregnant women, and patients whose bodies, through improper treatment, have been made receptacles for numerous drugs.

An excellent prognosis is a slightly moist skin; a good secretion of urine, 900 grammes or more per day, a moderate amount of albumin and jaundice; a quiet digestive system, which will allow of fluids being freely taken ; slight epistaxis ; a restful disposition and ability to sleep, and fever from 102° to 103·5° or 104° F. (38·9° to 39·7° or 40° C.) with gradual defervescence by the third day.

A fair prognosis is a drier skin ; 600 grammes of urine, albumin increasing but not exceeding 2·0 to 3·0 grammes per litre ; light jaundice ; slight attacks of black vomit which occur on the fifth day, or glosso-labial haemorrhages.

A serious prognosis is a dry skin ; high fever of 103·5° to 104·4° F. 39° to 40·2° C.) for two or more days ; considerable gastric disturbance from the onset ; early appearance of albumin which, although in small amount, is associated with a diminution in the quantity of urine, 400 to 600 grames ; much jaundice ; hiccough, fairly copious black vomit ; epistaxis, glosso-labial haemorrhages ; tendency to become delirious ; feeble and thready pulse.

A very grave prognosis is the above with greater tendency to copious black vomit and haemorrhages ; hiccough constantly recurring ; violent delirium ; pronounced icterus and failing renal system ; symptoms of collapse and subnormal temperature, or a history of temperature falling on the third or fourth day and followed by a sudden rise to 104° F. (40° C.) with complications such as abscesses of the parotids or pneumonia.

MANAGEMENT AND TREATMENT

The most important points in the management of yellow fever cases are nursing, absolute rest in bed, restriction of all nourishment for the first few days, the use of alkaline waters and the cautious administration of drugs. You must make yourselves responsible for the entire management of the case, and should : —

1. Obtain as full a history of the onset as possible, and of the patient's habits, temperament, etc.

2. Examine your patient thoroughly, and do not postpone the examination. Yellow fever is a disease which runs too rapid a course to permit of any delay.

3. Examine a fresh specimen of urine. If you have been summoned within the first twenty four hours it will probably contain no albumin. Give orders to preserve all the urine passed by the

patient and arrange that the necessary covered jars are provided so that the full quantity for the twenty-four hours can be kept. See that a special jar is always ready for holding the last urine voided.

Give orders that the patient shall urinate before the bowels move or before a clyster is given. This is important, as later on when the amount of urine is decreasing, much urine may be lost in the bed pan. Examine the urine for albumin and test the reaction at least twice daily, until convalescence is established. You have to ascertain the time when the albumin appears and afterwards the information obtained from the daily examination of the urine as to the amount of albumin, urea, and the reaction will be one of the most important guides to the prognosis and management of the case. Albumin is generally present in this disease, appearing between the second and third day. In severe cases it may be found in the first twenty-four hours. In very mild cases it may only appear for a few hours. In some cases it is never found throughout the course of the disease.

4. Obtain a trained nurse. Too much care cannot be given to the patient, and severe cases will require two trained nurses. In default of trained nurses your patient will suffer, but it will not be your fault.

5. Give instructions to have the temperature, pulse and respirations taken and recorded every two to four hours, and check the results by doing it yourself at least twice in every twenty-four hours.

6. Order the patient to be screened both day and night. Do not fail to insist on the utmost care being taken to prevent Stegomyia feeding on the patient. The lives of all non-immunes depend in a great measure on this precaution being observed. Even if you have not made a positive diagnosis, so long as there is the least suspicion that it may possibly be yellow fever, your duty is to insist on the screening. The screening saves your patient from being annoyed by mosquitoes and is an educational factor to the general public. If it is a case of yellow fever, and over the fourth day of the disease, the patient is non-infective and screening is not absolutely necessary.

7. The various rules and regulations of the Sanitary Departments should be complied with.

Nursing.

If trained nurses are available, give the instructions about urine, temperature, pulse, medicines, etc., in writing. See that suitable temperature charts are provided. Failing trained nurses, willing volunteers must be instructed, but in severe cases the lack of skilled nursing cannot but militate against the ultimate outcome. A yellow fever case requires to be nursed even more intelligently than a typhoid case. The disease is rapid; it is a question of hours, not of days, and when the critical moment arrives, unless you are on the spot, much valuable time will be irretrievably lost. I speak from experience, as my colleagues and myself are heavily handicapped by lack of trained lay nurses in treating patients in private houses. Many a time have I had to acknowledge that the public hospital yellow fever patient was better provided for than the richer private patient. The volunteers are willing, but the facilities for nursing are absent and impossible to obtain.

Until well on the road to recovery the patient should never be left alone. Visitors must be prohibited, and only those actually in attendance on the case should enter the room.

The room should be large and airy, and the bed should be so arranged that day and artificial light will not cause annoyance. Photophobia is frequently a most distressing symptom. Ventilation is necessary, but care should be taken that the patient is exposed to no draught. The bed should be narrow and have good springs; the mattress should be covered with a rubber sheet. The bedclothing should be light. A sheet is usually sufficient.

The necessary bed pans and urine-bottles should be procured. A generous supply of bowls should be ready for catching the vomitus, and when black vomit appears these should be changed as quickly as possible. Ice and ice-bags are necessary, as is also a hypodermic syringe, previously sterilised and ready for any emergency, and a collection of ampoules of caffeine, spartein, strychnine and ether, and some tubes of ethyl chloride; cotton wool for the gums and nates; a cleansing solution for the mouth.

The patient should be made to understand that absolute rest in bed is necessary. If possible the faeces and urine should be passed in the bed pan and bottle. No excitement should be allowed, and if sleep can be obtained the outlook is all the better.

Purge.

As practically all cases of yellow fever have been in robust health until suddenly stricken with the disease, it is advisable to give a purge. Some doctors prefer castor oil, others fluid magnesia, various cathartic mixtures or large doses of calomel. I favour small fractional doses of calomel followed by sodium sulphate. The latter is an excellent diuretic and, with the calomel, often allays the acidity of the stomach. After the preliminary purge, it is inadvisable to give any more, as we should irritate the bowels as little as possible, and enemata will answer the patient's needs.

Diaphoresis.

When the purge has been taken, a hot mustard foot-bath or hot pack should be given. In order to induce free diaphoresis hot demulcent drinks should be taken Free perspiration for a short time is comforting to the patient and markedly relieves the congestion Care should be taken to avoid the symptoms of excessive sweating which are so graphically described by Carroll.* Sweating should be confined to the first twenty-four hours of the disease.

Enemata.

It is an almost universal custom to order clysters, and they vary as to amount, number and composition. One large one every twelve sometimes every eight hours is sufficient. Some doctors use solutions of boracic, others eucalyptus, sodium chloride or sodium sulphate. I prefer the two latter, using about 600 c.c or 1 litre of solution to be injected slowly and retained as long as possible.

The eucalyptus and boracic are supposed to be excellent, but I believe that their virtues rest in the mere cleansing of the lower bowel and the sensation of comfort imparted to the patient. The temperature of the clyster should be usually about 65° to 70° F. (18·5° to 24° C.). Some prefer to use ice-cold enemata, but they generally cause the patient discomfort and do not appear to reduce the temperature very much.

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* Carroll, James, *loc. cit.*

Nourishment.

Prohibit all nourishment for the first three or four days. The patient is able to do without any food, and his stomach obtains the needed rest. Some doctors give small quantities of milk, but it only increases the tendency to nausea, and is almost invariably vomited.

Alkaline drinks.

Vichy, source Célestins, is universally used for quenching the thirst of the patient. It is an excellent alkaline diuretic water, and is readily taken. Because of the burning thirst the patient will be eager to consume large quantities of this water, and he should be encouraged to drink three to four litres daily. He is liable to drink to excess and over-distend his stomach, causing vomiting. It is therefore advisable that he should be given definite quantities at regular intervals; a cocktail glassful every quarter of an hour should be offered him; this he can sip or drink at once. Should he fall asleep and miss part of the allowance larger quantities should be given on his awakening. The great aim being that at least three litres should be imbibed daily. The amount of urine increases at once, the kidneys are well flushed, and the acidity of the stomach corrected. The Vichy can be given iced or at room temperature. You will find the majority of patients prefer Vichy as cold as possible. A few patients dislike Vichy, and in that case Apollinaris, Mattoni, or any other carbonated alkaline water can be substituted. If the water is very gaseous, it is advisable to allow some of the gas to escape in order to prevent much gas being retained in the stomach, inducing eructations and possibly hiccough. In the absence of Vichy or other alkaline water, you can alkalinize the ordinary drinking water with bicarbonate of sodium. Some doctors use the juice of fresh limes mixed with potassium bicarbonate to render the drink neutral. Occasionally this can be given in the first forty-eight hours, but the alkaline water does not upset the stomach, and larger quantities are drunk, which is what we aim at.

Venesection.

As a rule this mode of treatment is not necessary, but very plethoric subjects are considerably eased by the removal of 100 to 150 c.c. blood. I have had patients implore me to repeat the venesection. In the past, patients were almost exsanguinated, hence blood-letting fell into disuse, but where the congestion is intense, the carotids visibly pulsating and the veins engorged, venesection is indicated in the robust, and is often the only remedy which will relieve the splitting headache It is of no use in the later stages of the disease.

Ice-bags.

For the cephalalgia, applications of ice are of use, and especially when applied in the helmet ice-bag which covers the entire head from the forehead to base of the occiput. A Leiter's coil can be used An ordinary rubber air-cushion or a hot water bag filled with ice-water affords great relief to patients who will not use the ice-bag For the vomiting an ice-bag can be used. An excellent plan is to use a spray of ethyl chloride. This frequently checks the tendency to vomit and can be instantly applied.

Spongings.

These are most excellent and should be used according to the degree of fever and the reaction exhibited by the patient. Spongings should be given every two hours if the temperature exceeds 101° F (38·3° C.); if over 103° F. (39·4° C.) every hour. The temperature of the water should be from 75° to 60° F. (24° to 15·5° C.) or even lower

Baths.

Cold baths are valuable if the temperature is over 104° F. (40° C.) but are only advised if they entail no undue exertion on the part of the patient. A sufficient supply of water, the necessary full length bath and assistance, etc., are usually wanting. Hence spongings are more favoured.

Cold packs.

Are useful, if assistance is limited.

All of these applications are grateful to the patient, and if properly given are of great help during the fever period. It is hardly necessary for me to tell you that you will have to initiate all these forms of treatment in order that you may determine if a suitable reaction is being obtained, and to teach the nurses. The spongings or baths render good service in keeping the skin of the patient clean, and help to lessen the formation of boils.

Care of the gums.

The gums and mouth should be most carefully attended to. The gums are especially prone to become spongy and to bleed. Inflammation of the parotids with abscess formation is a frequent complication. The use of the tooth brush should be interdicted and the teeth cleansed with tooth-powder and pledglets of cotton wool. An antiseptic mouth-wash should be used four or six times a day to harden the gums and relieve the filthy taste in the mouth.

Many cases will require very little more treatment, and drugs should not be given unless absolutely necessary. Your main care should be to supervise the nursing, absolute rest of the patient, starvation, the early purging and sweating, the reduction of the fever by cold spongings or baths, the use of alkaline drinks, enemata, ice-bags to the head and cleansing of the mouth and gums. It will require all your vigilance to direct the nursing and management of the case and to check the condition of the patient by his temperature, pulse rate and the action of the kidneys.

Yellow fever is a disease which cannot be cured by drugs, and while employing symptomatic treatment you should endeavour to avoid causing any irritation to the stomach.

In addition to Vichy many doctors prescribe fluid magnesia with benzoate of soda, bicarbonate of soda either in cachets of 1·0 to 2·0 grammes every three to four hours, or in solution of 1·5 to 2·0 grammes to 100 c.c. of water, giving 30 c.c. every hour. I prefer the cachets, as they are not so disagreeable to take. If abundant alkaline water is drunk, and sodium carbonate or magnesia is given in addition, the acid reaction of the urine will gradually give place to a neutral or alkaline reaction. This is a favourable indication.

Kidneys.

Should the urine, despite the drinking of abundant alkaline waters, rapidly decrease in amount and failure of the renal system appear imminent, no time must be lost. Caffeine citrate is a cardiac tonic and acts beneficially on the kidneys. It is easy to administer in the form of hypodermics, and should be given in preference to other stimulants. If the kidneys fail to respond, it is justifiable to give theobromine, 2·0 to 3·0 grammes in the course of the day, usually in cachets of 0·3 to 0·5 gramme, or in the form of an emulsion of theobromine, benzoate of soda, and sufficient gum. Hot air-baths, hot hip baths, linseed poultices to the loins or a localised hot packs can be tried. Carroll[*] suggested that a hypodermic or rectal injection of urea (1·0 gramme) might be given if anuria is impending.

In order to ascertain if the kidneys are freely functioning the patient should be trained to use the bottle every two or three hours. In this way we can gauge the reacting power of the kidneys. It sometimes occurs that the urinary bladder becomes paralysed and therefore if no urine is being voluntarily voided, the patient should be catheterized. Retention of the urine is usually a bad omen.

Alcohol in the form of good sound champagne is generally used. It is a useful stimulant and is well taken by the patient. A good Rhine wine is preferable. Vichy should be added to the wine. Do not use red wine, as, should the patient vomit, the mixture might be mistaken for black vomit.

Irrigation of the lower bowel with sodium sulphate should be tried. Never despair of your case so long as the urine continues to flow. It has been suggested to perform decapsulization of the kidneys; it may be tried, as the pathological processes are not so intense that all the kidney substance is destroyed.

Vomiting.

This is a most common symptom. It is usually present in the first few hours after the onset, but with abstinence from all food and carefully regulated drinking of Vichy it generally ceases. In alcoholics or patients who have previously suffered from gastritis, vomiting is apt to be a marked symptom of yellow fever. Cease giving any liquids for a couple of hours and then cautiously begin with spoonfuls of iced Vichy. If the patient complains of thirst let him wash his mouth with strong black tea or acid lemonade, but do not allow him to swallow. A sinapism of mustard or an ice bag to the epigastrium is useful. Chloride of ethyl applied in a fine spray to the epigastric region when a paroxysm of vomiting is about to occur, very often stops the attacks. No medicines must be given by the mouth for some hours after vomiting has ceased. Give the alkaline treatment in the form of small clysters of bicarbonate of soda. Sucking cracked ice is useful in nausea.

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[*] Carroll, *loc. cit.*

Lavage of the stomach has been tried, but is rarely successful. Cocaine, chloroform, tincture of peppermint are advised by many, but nothing succeeds like absolute rest, and the doctor must see that it is enforced, as continued vomiting invariably brings on black vomit and wears out the strength of the patient.

Hiccoughs.

Are apt to be troublesome. In the early stages a little fresh, iced champagne fully charged with gas works wonders. In the later stages the hiccoughs are signs of ammonaemia.

Black vomit.

The treatment for ordinary vomiting should be tried. Iron has not proved of much value nor has ergot. Adrenalin by the mouth has appeared to be of benefit. All fluids should be stopped so that the adrenalin can have time to act in an undiluted state. Give 20 to 40 drops in a teaspoonful of water. Have plenty of bowls to catch the vomit, and if possible do not let the patient see it. Many patients will not notice the colour or will believe it to be bile. Do not confound haemorrhages from the gums or posterior nares with black vomit. The recumbent patient may swallow the blood and later on vomit it; this is apt to occur in the first day and will create a wrong prognosis.

Bowels.

The ordinary enemata are sufficient to keep the bowels open. Diarrhoea is liable to occur in the haemorrhagic stage, either from blood in the stomach or bleeding from the congested duodenum. The evacuations are tarry and intensely foetid. Large clysters given very slowly should be administered.

Delirium.

For mild or intermittent delirium, apply an ice-bag or pillow to the head, and enjoin absolute quiet in the patient's room. A hot bath is of service. Use as little restraint as possible. In violent delirium, maniacal patients may have to be tied down. The condition is hopeless. Bromides and chloral have been recommended.

Heart.

For cardiac weakness in the later days caffeine is best. Spartein and strychnine are of use. Alcohol is of value. Great care must be taken to prevent collapse, and especially when the bradycardia is pronounced. Ether is useful, especially if collapse is threatened through attacks of vomiting.

Complications.

Inflammation of the parotid glands is not a very rare occurrence and is a most distressing complication. The prognosis is fair. Applications of ice are most comforting. If abscesses form they must be opened. The swelling is generally bi lateral. Ichthyol proves of service in some cases.

Other glands may inflame and suppurate. The subjects generally have had severe and prolonged attacks of yellow fever with intestinal complications. Pneumonia is a most grave complication.

Furunculosis is very troublesome during convalescence, and several crops of boils may occur.

Management of convalescence.

Recovery must be slow and the patient will require careful treatment. In a mild case nourishment can be given even on the fourth day. Milk mixed with lime water or Vichy, or toast-water, rice and barley-water should be given in small amounts, and if well retained the amount can be increased by degrees. Chicken jelly, raw eggs and fish should constitute the diet of the patient for the first week of convalescence. In severer cases great care is necessary to avoid bringing on vomiting. If the temperature is below 102° F. (38·9° C.) give the nourishment in small quantities. The patient must not be trusted to control his appetite; he is ravenous and will gorge himself unless prevented. Fatal relapses are usually due to indiscretions in diet. As long as the pulse is slow great caution must be exercised and exertion avoided.

The icterus should be treated by sodium sulphate waters. The kidneys rapidly regain their normal state. Convalescents are usually very erotic. They should be warned of the dangers incurred by early intercourse, while their heart muscle is still degenerated.

A few more words and I have finished. No reference has been made to any of the coal-tar products. I do not use them. If you pin your faith to these antipyretics you are only creating trouble for your patient. The older and the newer compounds have the same depressing action in yellow fever.

Educate your clients never to neglect a case of yellow fever. Half the battle is gained if the patient is in bed and under treatment within the first few hours of the onset. I cannot do better than give you the opinion of Touatre*: 'At the onset of the infection the patient can be succoured, but what can be done when the organism is poisoned, when the toxin has already affected the hepatic cells and the renal parenchyma, and when the mucous membranes are bleeding? The physician is practically disarmed, for such lesions are nearly always fatal. It is during the first days of the disease that the physician must act. When black vomit has come to darken the situation, we can yet save some patients, but are much better prepared to prevent the occurrence of black vomit than to cure it. The first and most indispensable thing for success in the treatment of yellow fever is that it be begun as soon as the disease has declared itself.'

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* Touatre, Just.; Yellow Fever. New Orleans, 1898.

THE MOSQUITOS OF THE AMAZON REGION

BY

R. NEWSTEAD, M.Sc., A.L.S., ETC.

AND

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(Plate XI)

Reference has already been made in this report to the general breeding-places of the various mosquitos encountered in Manáos, Brazil (pp. 17-22). Dr. Thomas has also published a short preliminary note* on some of the Culicidae found by him in Iquitos, Peru; no details, however, are given, and the exact identifications of the species at that period were not ascertained.

The list of species recorded in this memoir embraces all the commoner kinds and a few that are new or noteworthy. It is highly probable, however, that other mosquitos await the discoverer in a region so rich in insect life, but it has been impossible hitherto, owing to pressure of official work, to extend the researches in this department of Zoology farther afield.

Cellia argyrotarsis (R. Desvoidy), fig. 2.

This is undoubtedly a 'domestic' species, occurring sparingly in the dwellings of man near the swamps both in the town and its environs. It is much less common than its near ally *C. albimana*, from which it is readily distinguished by the absence of the black ring around the base of the terminal segment of the hind tarsi.

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* Lancet, September 8, 1906, p. 668.

Cellia albimana, (Wiedemann)

This is the Anopheline *par excellence* of the Amazon region. It is characterised by the presence of a black ring at the base of the last segment of the otherwise white hind tarsi. It is also a decidedly 'domestic' species, and is both intra-urban and extra-urban; but is naturally most abundant in the swampy regions. It bites in the day time as well as at night; but is rarely met with in the houses until after sunset.

It is the common practice among the natives of Brazil and Peru to sink tubs or barrels in the ground at or near the margins of the swamps. These receptacles fill rapidly with clear water which is used for drinking and washing purposes, and eventually become the chosen breeding place for this Anopheline. This habit is all the more remarkable seeing that the water attains a depth of three to four feet with a total absence of the higher forms of aquatic plants. We have also to record the capture of this mosquito at Itacoatiara and Breves on the Amazon River, East of Manáos; and at Santo Antonio on the Madeira River about 600 miles S.W. of the same city.

Stethomyia nimba, Theobald

One example taken at Taruman Waterfall about 18 miles N.W. of Manáos, July, 1906. Seen also in the swamp near Pensador. This curious Anopheline has hitherto been recorded only from British Guiana and Pará.

Megarhinus separatus, Arribalzaga

Several examples of this handsome mosquito were taken at Manáos, Brazil, during the month of July, 1906. They all occurred in a reclaimed portion of the forest surrounded by swamps. These mosquitos could not be induced to feed on man; and the larvae were not discoverable.

Janthinosoma musica, Say

Two specimens, both females, were captured in Dr. Vigil's house at Iquitos, Peru. The residence was surrounded by low shrubs and bananas far from any natural collection of terrestrial waters; but innumerable puddles and road-side gutters were near at hand, so that the insects may in all probability have bred in some of these.

Stegomyia calopus, (Meigen)
Stegomyia fasciata (Fab.)

This mosquito is present all the year round; there seems to be no marked seasonal prevalence. As already stated (ante, p. 17), the larvae occur everywhere, in any receptacle which will hold water for a few days. Great numbers, however, were found in large puddles in the open, in association with Anophelines and other mosquito larvae, although there were houses with suitable receptacles within 50 feet of these habitats. They occurred also in two large deep collections of terrestrial water averaging 3 feet in depth, situate at the upper reaches of two Igarapés within the limits of the city (No. 9 on map). On one occasion, swarms of larvae were found in a barrel of water containing the macerated and putrid remains of a number of frogs upon whose carcases they seemed to have been thriving.

The females bite freely both by day as well as at night, though they are naturally much more noticeable in the day time, and they are most persistent in following man from place to place whether in sunlight or shadow.

Being desirous of confirming the experiment conducted by Mr. Theobald* regarding the viability of the ova of this mosquito after long exposure to dry atmospheric conditions, two small consignments of eggs were forwarded to Liverpool in the year 1906. The first batch was laid on moist white filter paper; these were dried in the air and subsequently placed in a desiccator with chloride of calcium for 24 hours, and finally transmitted to England in glass tubes, tightly corked. The following data gives the results of this experiment:

September	9-11.	Eggs laid at Manáos, Amazon.
October	26.	Eggs reached England. Placed in water at a temperature of 23° C.
,,	27.	12 larvae hatched during the previous night and 1 after 12 hours immersion.
,,	28.	Larvae began moulting.
,,	30.	All larvae completed first moult.
November	4.	Larvae pupated.
,,	7.	First imago, a male, hatched. This example lived for 6 days.
,,	8.	A male and female hatched.

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* Mono. Culicid., Vol. III, p. 6.

The breeding jar was kept in an incubator at an even temperature of 23° C. For the greater part of the time the insects were in complete darkness; but a little light was admitted occasionally during the day.

SUMMARY

Eggs remained dormant and practically dry 45-47 days.

LIFE-CYCLE

Eggs. Incubation period	6-12 hours
Larval stage	9 days
Pupal stage	3 days
Complete cycle	12-13 days

Culex fatigans, Wiedmann

Abundant everywhere in the towns and suburbs, both at Iquitos, Peru; and at Manáos, Brazil. It breeds in large numbers in almost any collection of water, but the larvae were less common in the water of flooded cellars than were those of *Mansonia titillans.*

Mansonia titillans, (Walker)

This species is as common as *Stegomyia calopus* in Iquitos, Peru. Its breeding-places are confined chiefly to the pools and puddles scattered throughout the town; it was most abundant, however, in pools along the river banks. The specimens here recorded were all captured during the first three months of the year 1906.

Examples of the adults were caught attempting to bite persons in well lighted rooms and outside in shady places, especially between the hours of 7 a.m. and 9.30 a.m., and again in the early afternoon, but it was only at dusk that swarms of these mosquitos appeared, attacking vertebrates of all kinds. On board the ships, alongside the Muelle, the walls of the cabins were at times black with them, and they were a source of great discomfort, boldly attacking everyone and biting most viciously. The occupants of the houses along the river bank were attacked to such an extent that the residences at times became almost untenable. In the centre of the town, fortunately, the nuisance was not so great, due chiefly to the absence of breeding-places.

In Manáos, it is found in the suburbs of the city at the pumping-station, and in the neighbourhood of some of the Igarapés. It was especially troublesome during 'high river' in the year 1908, when many of the cellars of the houses and offices situated along the low-lying land by the river front were filled with water. This Mansonia bred in the collections of water thus formed in large numbers, but the larvae were not found where the water had been oiled. The nuisance caused by these mosquitos was very great, and it was almost impossible to have even the early meals—breakfast and lunch—in peace.

Mansonia pseudotitillans, Theobald

One example, a female, agrees for the most part with the description given by Theobald,* and is apparently referable to this species. It differs, however, in having a few *short* clavate lateral scales on the wings; but these are much shorter than in *M. titillans*, and very few of them are present. Unfortunately the anterior tarsi are wanting, so that the record must, for the present, remain a somewhat doubtful one.

Habitat:—Iquitos, January 13th, 1906. The capture was made in the afternoon on board the SS. 'Napo' while lying alongside the Muelle. This mosquito has not hitherto been recorded from Peru.

Mansonia longipalpis, n. sp. (Newstead and Thomas)

(Plate XI, figs. 5, 5*a*)

Female. Palpi long (fig. 5). Thorax clothed with golden ochreous scales; posterior region black. Abdomen blackish with lateral patches of creamy-white scales. Legs distinctly speckled; hind tibiae white with a black band. Wings with a double dense black bar at base.

Length, 4-4·5 mm. Length of detached wing 5 mm.

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* Mono. Culicid., Vol. II, p. 177.

Head scales. Upright forked, black; narrow curved, pale ochreous with golden reflections; flat, creamy white.

Palpi (Plate XI, fig. 5). A little more than one-third the length of the proboscis; tips clothed with white scales; the remaining scales mixed creamy white, pale ochreous and blackish.

Proboscis with an incomplete central band of creamy-white scales; the scales on the ventral surface of the pale area black, those on the remaining portion of the proboscis blackish with pale ochreous ones intermixed.

Thorax with golden ochreous scales intermixed with a few black ones in front; those over the insertion of the wings very long and whitish; region in front of the scutellum semi-nude, blackish-brown with a well defined median group of black scales; pleurae clothed with flat (some almost spindle-shaped) creamy white scales.

Abdomen. Blackish with a few creamy-white scales at the base of the segments; and lateral sub-triangular patches of creamy-white. Apical margins of all the segments with a fringe of very long golden brown hairs.

Legs black or brownish-black; conspicuously speckled with bright ochreous yellow; last segment of the anterior tarsi with an inconspicuous band of dull ochreous; *mid tarsi*: apex of 2nd, basal two-thirds and apex of third, and the whole of the 4th and 5th ochreous white; *hind tarsi*: apex of 2nd, the whole of the 3rd; base and apex of the 4th and the whole of the 5th ochreous white.

Wings densely clothed with blackish and creamy white scales, the former predominating; base of costa, sub-costa, and first longitudinal vein, black. Fringe smoky-grey. Outstanding scales (Plate XI, fig. 5*a*) more than twice the length of the broad spathuliform ones, and considerably longer than those in *Mansonia titillans* (fig. 4*a*).

The distinguishing features of this insect are the abnormally long palpi, the colour of the hind tarsi and the great length of the outstanding scales of the wing. It is a large and very characteristically marked insect, and may from the character of the palpi form the type of a new genus.

Habitat: —Four females, all taken at a large residence at the pumping station standing at a considerable elevation and surrounded by woods and swamps. It is one of the coolest spots in the immediate neighbourhood of Manáos. They were all taken between the hours of 5 and 5 30 p.m. during the month of August, 1906 and 1907.

Uranotaenia geometrica, Theobald

(Plate XI, fig. 3)

This very handsome mosquito was commonly found in the neighbourhood of swamps and large puddles within the city of Manáos and also in its environs. It was usually associated with *Culex fatigans*, *Cellia albipes*, and occasionally with *Stegomyia calopus*. At Iquitos, Peru, it was also common in the swamps near the villages of San Juan and San Miguel.

The majority of the specimens were captured out of doors; but it occurred, not infrequently, in dwelling-houses and also in the Hospital da Portugueza Beneficente, Manáos. For details regarding the distribution of this mosquito in the latter locality, see map opposite p. 54.

Goeldi in his delightful memoir* makes no reference to this insect. The type, however, was caught by Dr. Lutz at Cubatao, near Santos, Brazil. Dr. Lindenberg† also records this species from Manáos; but there is apparently no previous reference to its occurrence at Iquitos, Peru.

Uranotaenia pulcherrima, Arribalzaga

This beautiful little species occurs freely at the swamps along the Igarapé Castelhana, especially so in the one by the old cemetery, Manáos. The locality is a new and hitherto unrecorded one.

Uranotaenia lowii, Theobald

Habitat:—Flores and Pensador swamps, near Manáos. It occurs also within the limits of the city at the Cearense swamp and near the Power House of the Electric Tram system. Specimens were captured during the months of July and August, 1906, 1907.

Neomelaniconion chrysothorax, n. sp., (Newstead and Thomas)

Anterior half of thorax densely clothed with golden scales. Abdomen brown to brownish black with pale fringes. Legs bronzy brown or smoky brown (in subdued light); femora paler but black apically on the dorsal surface.

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* Os Mosquitos no Pará.

† Mosquitos do Brazil.

Female. Head scales: Upright forked ones pale ochreous to pale golden yellow in front, those on the nape black; flat scales pale ochreous, those on the lower portion of the head dusky white.

Thorax dark brown, clothed to a point near the insertion of the wings with large narrow curved golden bronzy scales. Posterior portion, with a narrow median black line and a central patch of minute narrow scales on the otherwise bare integument; posterior angles of meso-thorax with numerous long hairs; and there are many similar hairs on the scutellum.

Abdomen. Scales dark and pale brown, presenting a faintly freckled appearance under the microscope, the dark scales preponderating; apical margins with pale ochreous scales, giving the segments under a low magnification a faintly banded appearance, but this characteristic disappears under a higher magnification. Apical marginal hairs, whitish; sides, with lateral pale angular basal spots. Venter with distinct broad pale apical bands.

Legs coppery brown; femora whitish beneath.

Wings strongly hyaline, scales uniformly pale brown. Length 2-2·25 mm.

Male. Closely resembling the female; but the thoracic scales are slightly brighter or more golden, and the apical bands on the abdomen are also more distinct.

Habitat:—Iquitos, Peru. Several specimens caught in the centre of the town, but within 250 yards of swamps; February and March, 1906. Examples were also taken in the suburbs of Manáos at the old Pumping Station; at Flores, Pensador; and at the swamp near the Portuguese Hospital, June and July, 1906-1907. It would appear, therefore, that this insect may be met with from February to July, and in all probability at other seasons also.

One of the marked characteristics of this mosquito is the sudden line of demarcation between the golden scales of the anterior half of the thorax and the dark semi-nude portion behind the insertion of the wings.

Limatus durhami, Theobald

A single female of this well marked species was taken at Iquitos, Peru, March, 1906. It occurred in a dwelling-house situate at the margin of the very extensive swamps in the neighbourhood of the

Nona' Spring. It is noteworthy that the specimen in question was caught while biting its captor; and that there were no wild-pines (Bromiliaceæ) anywhere in the neighbourhood. This record is an interesting one, as it is a new addition to the Culicid Fauna of this region.

TABANIDAE

We add here the records of the capture of two species of Tabanidae. No efforts were made to collect these insects, as they require searching for in localities beyond the limits of the districts in which the work of the expedition was conducted.

Erephopsis brevistria, Lutz

Two examples, both females, were captured near Manáos at the Taruman Waterfall, 25.VI.06. They agree best with the description given by Lutz;* but the examples are rather 'worn,' so that it is somewhat difficult to fix all the specific details.

Lepidoselaga lepidota, (Wiedemann)

This common and widely distributed species is abundant in all suitable places between Manáos and Iquitos, coming on board the vessels in the river between these two places. It is a very striking insect, and owing to the sudden line of demarcation of the black wing markings it is known generally to Europeans as the 'short jacket'. In the immediate neighbourhood of Manáos it is commonest during the dry season, May to December, and is a persistent and vicious biter.

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* Tabaniden Braziliens. Zool. Jahr. Supp. X., 1909, p. 643.

EXPLANATION OF PLATE XI.

Fig. 1.—*Stegomyia calopus.* The carrier of Yellow Fever. × 10, and natural size, about. (Page 143.)

Fig. 2.—*Cellia argyrotarsus.* × 10, and natural size, about. (Page 141.)

Fig. 3.—*Uranotaenia geometrica.* × 10, and natural size, about. (Page 147.)

Fig. 4.—Head of *Mansonia titillans.* × 10. (Page 144.)

Fig. 4*a*.—Wing scales of *Mansonia titillans.* Magnified. (Page 144.

Fig. 5.—Head of *Mansonia longipalpis*, n. sp. × 10. (Page 144.

Fig. 5*a*.—Wing scales of *Mansonia longipalpis.* Magnified (Page 145.)

[illegible]ANATION OF PLATE XI.

[illegible] *[illegible] calopus.* The carrier of Yellow [illegible] natural size, about. (Page 143.)

[illegible] *[illegible] argyrotarsus.* × 10, and natural size, [illegible] (Page 141.)

[illegible] 3.—*Uranotaenia g[illegible]metrica.* × 10, and natural size, [illegible]

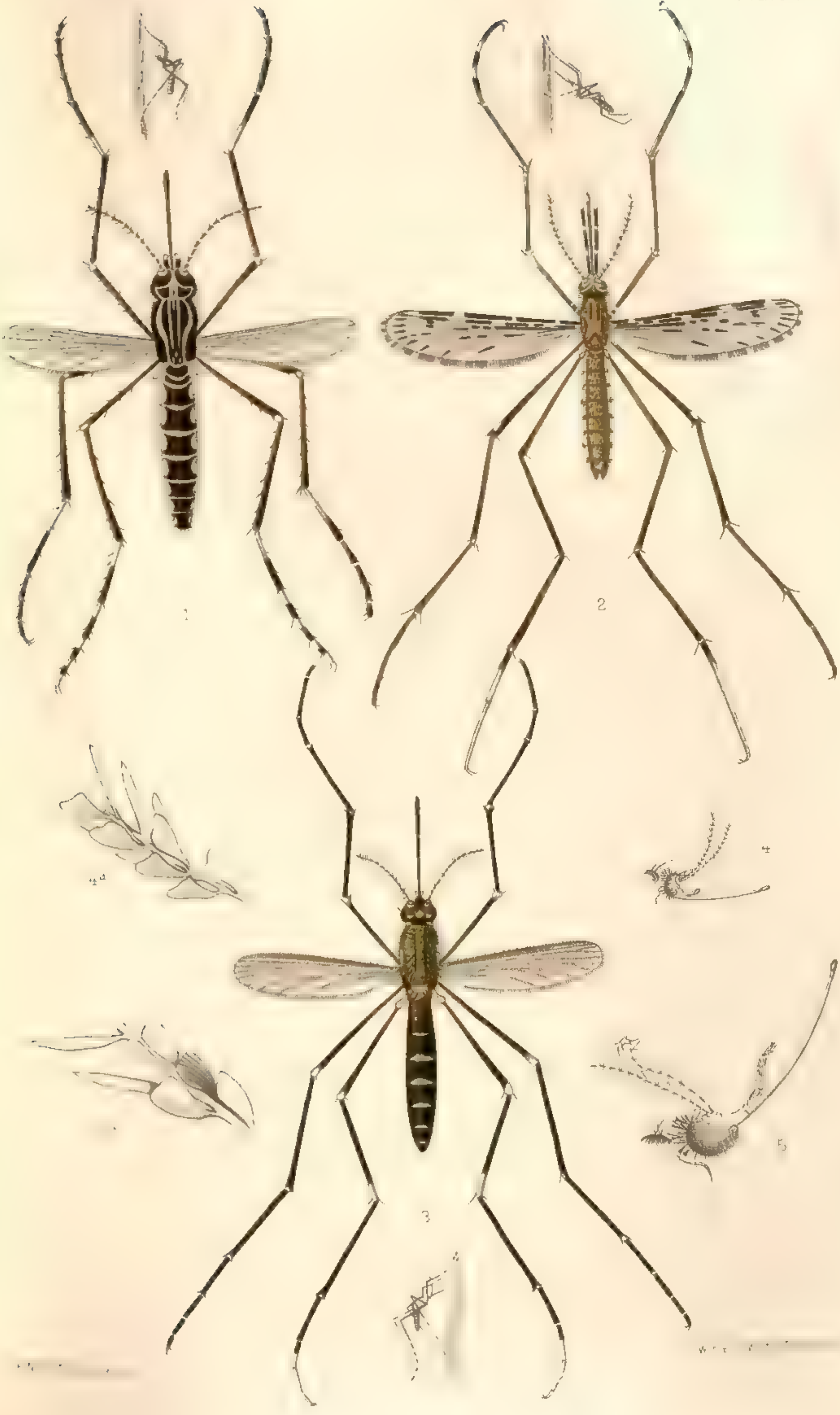
Fig. 4.—Head of *Mansonia titillans.* × 10. (Page [illegible]

Fig. 4*a*.—[illegible] of *Mansonia titillans.* Magnified. (P[illegible]

[illegible] *Mansonia longipalpis*, n. sp. × 1[illegible] [illegible]

[illegible] of *Mansonia longipalpis.* Mag[illegible]

Plate XI.



PARASITIC GRANULOMA:

A CONDITION ALLIED TO ORIENTAL SORE OCCURRING IN EGYPT

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PLATES XII-XV

Outpatient practice among the poorer classes in Egypt includes a variety of conditions not described in the English textbooks. One of these has presented itself with sufficient frequency to induce us to try and investigate it. It consists essentially of a chronic elevated patch or warty growth in the skin, sharply localised, and unaccompanied by other symptoms. Most of the cases we have so far seen have been structurally papillomata, others have consisted of flat skin-covered granulomata. We have described them as warty and flat forms respectively, and in both we have occasionally found 'bodies' of the same class as those described in Oriental sore. Clinically, however, these cases differ widely from the usual description of that condition, and we do not think they can be classed under that name as it is used at present.

Taking these two forms of the disease together, we have, so far, investigated clinically and pathologically ten cases of the nature of which we feel tolerably certain. Besides these, we have observed a number of similar ones in which some doubt existed, or of which full notes have not been kept. In addition to these, Professor Bitter and Dr. Dreyer, Bacteriologist and Assistant Bacteriologist respectively to the Egyptian Government, have each met with a case of a similar nature, in which the parasite was found. They have kindly permitted us to append their notes of these cases to the present paper. We desire further to acknowledge our indebtedness to Professor Bitter for allowing us to reproduce his excellent micro-photographs of the parasites seen in Pl. XIV figs. 11, 12, 13.

There is no doubt that this condition was equally common in the past, and the diagnoses show that there was less difficulty in distinguishing it from other diseases than in finding a suitable name for it. Among the names previously employed by various English and Egyptian members of the staff, we find 'papilliferous degeneration of skin'; 'chronic benign papilloma'; 'pseudo-epithelioma'; 'false elephantiasis'; 'hypertrophic dermatitis' 'lupus erythematosus exanthematosus'; 'granuloma of foot' 'partial ichthyosis'; 'parasitic growth'; 'fungous growth' 'fungating granuloma.'

Cases bearing these very varied titles seem, as far as we can gather from reports, and in some cases observation, to have belonged to the class described above. These names are interesting as showing the clinical features which struck a number of different observers in individual cases.

Incidence. In the ten cases we have selected, the age of the patients varied from eighteen to sixty, with an average of thirty; they were all males, and with the exception of two students, all fellaheen. Five of them were affected on the forearm or hand four on the legs or feet, one on the face. Half of them had single lesions, half multiple. The duration of the disease varied from six weeks to ten years; excluding the last figure they averaged six months.

Consequently, as far as we can conclude from so few cases, the disease, as we meet with it, is one affecting male fellaheen at any time in their adult working life, it may be single or multiple, and it affects the arms and legs about equally: the patients usually apply for relief about six months after the disease has begun.

We have never obtained any history of similar cases in the same village from which infection might have been acquired. Where infection is multiple, the lesions are usually near one another. This may be due to simultaneous infection, but more probably, especially where there is an interval of time between them, to auto-inoculation from scratching.

The chronicity of the condition is sufficiently well shown by the histories. The rate of progress varies a good deal in different cases, but is slow in all; the channel of infection is probably through the skin, for it appears almost exclusively on uncovered parts, i.e., the limbs and face rather than the trunk.

As regards the constitutional condition of the patients, they none of them gave any constant or important medical history, and their general health was clearly unaffected. In those cases in which we examined for enlargement of liver or spleen, it was absent.

1. WARTY FORM

These masses begin as small painless tubercles and may gradually attain a diameter of three or four inches. The skin round their edge is usually healthy, but it may be reddened from sepsis, or show a definite areola marked by loss or increase of pigment. They have a very definite, raised, even overhanging edge, and may rise nearly an inch abruptly from the level of the surrounding skin. The surface of the growth, by the time it is seen, is often ulcerated in whole or part, but typically is covered by hard whitish epithelium, and presents a warty cauliflower-like appearance. It is intersected by clefts lined with foul decomposing epidermis; indeed, where bacterial infection has occurred, it is possible to express masses of dead epithelium with pus from numerous apertures under the overhanging edge. In places where movement naturally takes place, as on the front of the ankle, some of these clefts deepen and appear as transverse fissures which extend through the skin and give rise to pain on movement. With this exception, the disease appears to be painless. There is occasionally some local oedema and enlargement of lymphatic glands, but these depend on secondary sepsis and are not an essential part of the disease.

Most of these growths become septic in course of time, and full of mixed infection. Most of their offensive smell and foul appearance is due, however, to decomposition of epithelial masses on the surface and in the clefts, outside the substance of the growths altogether. If they could be kept clean, which of course they never are, they would probably show a firm white mammillated surface, like the head of a raw cauliflower, to sight and touch.

A very striking feature, and one that marks them off at once from malignant growths, is the way in which they are confined to the skin, and hardly ever affect even the most superficial tendons An extensive growth on the dorsum of the foot scarcely affected the movement of the extensors of the toes. And it is quite easy after running an incision round them, to strip them like a scalp off the deep fascia, leaving a smooth surface on which grafts take readily.

An exception to this was Case VIII, in which the growth situated on the inner side of the elbow, had enveloped the ulnar nerve and invaded the internal condyle for a short distance. But this growth had been present for ten years, and the ulnar nerve, which ran through it much as the spinal accessory runs through tubercular glands, was easily freed, and covered with a skin graft. It retained its motor and sensory functions unimpaired. The fact that the growth could completely surround a nerve for a distance of some two inches for a period of several years, and leave it functionally active and sufficiently well nourished to take a skin graft on its surface, is very good evidence of its sharp limitation and innocent character.

II. FLAT FORM

So far we have only seen two instances of this variety as against eight of the preceding. They both occurred in students of the higher schools, one on the face and one on the forearm, and had been present for four and twelve months respectively. In appearance they were flat pink patches, raised one or two millimetres above the surrounding healthy skin, covered with thin epidermis, sharply limited, painless, soft, and freely moveable on the deeper structures.

Their essential identity with the preceding form is suggested by the fact that they contained similar intracellular parasites. It is possible that the apparent differences are due to external causes. Both these cases were in men of the educated class, who kept the growths clean and protected from irritation. It is possible that, if they had been situated on the bare and dirty limbs of fellaheen, exposed to constant friction and bacterial invasion, they might have shown the same proliferative changes which are so marked in the papillomatous form.

The two cases recently described by Balfour* fall into this class, in which, in our experience, the parasites are present in much larger numbers.

Treatment. A very large variety of lotions and ointments have been used for these growths. They seem to have no effect, beyond slightly diminishing the sepsis.

In one case a determined attempt was made to treat a large patch by ionization with iodine. Under this treatment there was distinct improvement up to a certain point, and the size of the growth was measurably lessened; but after six weeks there was not enough change to make it worth while continuing.

For fellaheen especially—who cannot afford to spend a long time in hospital—the only treatment worth considering is that of excision of the whole mass, followed by immediate skin grafting. The growths can be readily stripped off the underlying fascia, and in spite of the sepsis usually present, grafts take fairly well. A good example of this is shown in Pl. XIII, fig. 8 (Case VIII).

CASES

Case I. Pl. XII, figs. 1, 2. Man aged eighteen, admitted January 21, 1907, under Mr. Richards. Six weeks ago he had swelling of both legs; a few days later small red patches appeared, which increased in size and turned brown.

On admission there is a brown circular raised patch, three inches across, divided by irregular furrows containing a yellowish-brown secretion, situated in front of the ankle and extending back past the external malleolus. A similar one on the internal malleolus of the right leg, and a smaller one above it. Two more on the dorsum of the left foot. The patches have a foul smell, and are surrounded by an inflamed area. There is a little oedema of both feet.

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* 'Trans. Soc. Trop. Med. and Hyg.,' Vol. III, No. 3, p. 107, January, 1910.

The feet were cleaned, and various patches were treated with salicylic, boric, and yellow oxide of mercury ointment respectively. Potassium iodide was given internally.

After twelve days in hospital there was no improvement, and he was discharged at his own request, unrelieved.

Pathological Report (A.R.F.)

Great overgrowth of epithelium, with a cellular and vascular granulation tissue, pushing its way upwards through and between the greatly hypertrophied papillae. No purulent process or appearance suggestive of Madura foot. It may be a simple chronic wart, but the character of the infiltrating tissue beneath reminds one of a specific infiltration.

Case II. Pl. XIII, fig. 6. Man aged thirty, admitted August 4, 1908, under Mr. Richards. Four months ago a small painless papule, without discharge, appeared on the outer malleolus of the left leg, and increased in size.

The mass is the size of an egg, oval, raised above the surface, hard, papillomatous. The small papillae are of a purplish colour, and between them the surface is covered by yellowish crusts. It is sensitive to touch, but not painful, even on pressure.

Similar smaller growths occur on the chest and the right second toe, dating two and four months respectively.

It was treated for 18 days with liniment and tincture of iodine, and showed great improvement, but did not disappear. The patient then left hospital at his own request.

Case III. Pl. XII, figs. 3, 4. A man aged twenty-five, admitted April 23, 1908, under Dr. Phillips, transferred to Mr. Richards April 29.

Ten months ago he noticed rough, red, warty, painless masses appearing in front of right ankle and on dorsum of left foot. As they grew older they became white and encrusted. Four months later they began to be painful. No similar case in the district.

Right foot. A square papillomatous mass 12 × 11 cm. raised 2 cm. from the surface, over the front of the ankle joint. It is divided by *three deep transverse clefts* extending *right through it*, and filled with foul-smelling epithelium. The surface is covered with epidermis, and has a low-set warty cauliflower appearance. There is no ulceration. It is surrounded by a margin 1-3 cm. broad, in which the skin is raised, smooth and glistening with some loss of pigmentation.

A similar oval patch 7 × 3 cm. is found over and behind the external malleolus. Movement of tendons unaffected.

Left foot. A similar patch, 12 × 7½ cm., covering base of four inner toes and adjacent dorsum. Another over tendo Achillis, 6 × 4½ cm., its lower margin level with malleoli. This has no cracks. Enlarged glands in groin on both sides.

Pathological Note (A.R.F.)

A case, in my opinion, of the same parasitic nature as the two others (I, II). The parasite may be:—*a*, a pathogenic yeast; *b*, a mycelial fungus, Botryomycosis.

Treatment. All the growth was stripped off the right foot, leaving a smooth grey surface, which was painted with iodine, and afterwards skin-grafted. It healed well, but in July small warty indurations began to form in the scar, suggesting recurrence.

The left foot was treated by ionization with iodine. It decreased in circumference from 27 to 25½ cm., became painless and the movements of the toes quite free. It then remained stationary.

He was discharged at his own request, July 11.

Case IV. Pl. XIII, fig. 5. A man of 38, admitted April 24, 1909, under Dr. Day for cirrhosis and ascites.

On the right hand is a growth extending over the metacarpals of the first three fingers, with a raised border. The lesion was of a year's duration. It presented itself as a circular area with a firm, raised edge, which was rounded, covered with skin, and painless. Manipulation of the lesion was freely permitted, although the man said that it sometimes pained him at nights. There was no ulceration to speak of. The centre consisted of a thin, wrinkled cicatrix, said to be due to cauterization. The lesion had commenced centrally, and extension had since been going on at the edges.

Naked-eye section of the margin showed it to be composed of a solid white mass of epithelium, the processes of which ran downwards in parallel fashion towards a very definite lower boundary.

Pathological Report.

Repeated search in films failed to show Leishman-Wright parasites. Bacteria were not infrequently met with. They were diplococci, very like pneumococci, and some short bacilli. Histologically it was a sore of papillomatous type.

Case V. A man aged 21. Admitted on March 17, 1909, under Mr. Richards. No venereal history in self or family; no similar growths in the village; has inquired from everyone.

The present masses appeared six months ago, and have been painful enough to prevent his working for the last three months.

On the left foot is a patch, three inches by two, on the outer side of the front of the ankle, raised a quarter of an inch from the surface, partly skin-covered, partly granulomatous, surface like a cauliflower, definite sinuous raised edge surrounded by healthy skin.

A second similar patch, four inches by two, rising abruptly half an inch from healthy skin, starts at the front border of the tibia, and goes round past the tendo Achillis. The edge of the growth overlaps the surrounding skin. Both masses have yellow scabs adherent to their surface in places, and smell foul. Inguinal glands enlarged, foot and leg oedematous. The growths were stripped off by Dr. Aly Bey Ibrahim, and immediate skin-grafting done.

Patient discharged cured soon afterwards.

Pathological Report (A.R.F.)

The tissue is essentially of papillomatous structure, the penetrating epithelial columns passing into an almost continuous zone of small-celled infiltration. On careful examination, this proves to be mononuclear in character. In films treated by Giemsa's stain, large numbers of bodies having the closest resemblance to the parasite of Kala-Azar (*L. donovani*) but doubtless of the species *L. tropica* are found, both free and in the interior of large mononuclear phagocytes.

Case VI. A man aged 60, admitted August 23, 1909. A year ago a swelling appeared on the forearm, near the wrist, and four months ago a smaller one came higher up.

Two rough raised patches with thick everted edges, covered with scabs, and containing maggots. They were excised, and he was discharged cured.

Pathological Report.

No Leishman-Wright parasites found in scrapings. A good deal of mixed bacterial invasion, which may account for their absence. I have no doubt the growth is a parasitic papilloma.

Case VII. A man aged 26, admitted March 31, 1909, under Mr. Richards. Nine months ago a number of small red lumps appeared near one another on the inside of the middle of the left forearm.

The growth is superficial, does not affect movements. Its edge and part of its surface are covered with skin. Treated by excision and grafting, and discharged cured April 24.

Pathological Report.

Search for parasites, so far, negative. A rich secondary microbial infection, which adds to the difficulty of the search. The margins are diffusely infiltrated with pus.

Second Report. Prolonged examination revealed the presence, in the films, of the same parasite (*Leishmania tropica*) as was discovered in a preceding case (No. V), but in very scanty numbers. These were only found free amongst the bacteria of secondary invasion. Although their form was identical with that of those previously found, the staining of their cytoplasm, and in particular that of their larger chromatin masses was defective.

Case VIII. Pl. XIII, figs. 7, 8. A man aged 45, admitted October 27, 1909, under Mr. Richards. His trouble began as a small boil ten years ago, and has increased since. No treatment. No similar cases in the village.

The right arm is occupied from the elbow to half-way up the humerus, and over the inner and front half of its circumference by a swelling, rising with an abrupt and overhanging edge from the skin to a height of $1\frac{1}{2}$ to 2 cm. The skin immediately round the edge has a dark pigmentation about 2 cm. broad, but is otherwise healthy. The surface of the growth is mammillated, with ulcerated patches where the skin has worn off the tops of the projections, and divided by skin-lined cracks containing a foul fluid. The upper part moves freely on the deep tissues, the lower is anchored to the condyle. The arm is fixed at an angle of 85° with scarcely any flexion or extension, but pronation and supination are free. No glands in the axilla. At the operation the upper part of the growth peeled easily from the deep fascia, lower down it surrounded, without invading the ulnar nerve, and involved the bone just above the internal condyle. This area—the only one where there was any deep invasion—was scraped out and packed with sulphur; the remaining surface was grafted; and in spite of the filthy condition of the arm, most of the grafts took well. The ulnar nerve, which lay bare and isolated beneath the grafts, preserved all its motor and sensory functions. He was discharged with a small sinus leading to the bone.

Pathological Report (A.R.F.)

Microscopical search in films, treated with Giemsa's stain, shewed no *Leishmania tropica*. Very numerous bacteria, diplococci, short streptococci, and an undetermined species of bacillus, were present. These, when present in large numbers, seem to determine the disappearance of the parasite.

Case IX. Pl. XV, fig. 9. A third year medical student, dressing for Mr. Richards, complained of two patches, one on his forearm, the other on his wrist, slightly elevated, flat, soft, pinkish, skin-covered, sharply limited, and freely movable. They had been present about a year. They were both excised under cocaine-adrenalin anaesthesia.

Pathological Report (A.R.F.)

The skin over the central portion of the lesion shows only slight thickening. There is beneath a dense, almost homogeneous mononuclear cell-infiltration; in two places, degenerated foci occur, in which numerous remains of cell nuclei are visible, but no bacteria. Films were not made from this case, but the characteristic parasite was found in sections, though not plentifully, in the larger mononuclear cells of the subcutaneous infiltration.

Case X. A student of the School of Law was admitted under Mr. Richards, with a patch on the cheek immediately below the left eyelid, measuring one inch by five-eighths. Flat, soft, pink, smooth surface, covered by thin unbroken skin. The skin around was quite healthy. It had been present for the last four months, and had gradually increased in size.

It was excised by Dr. Aly Bey Ibrahim.

Pathological Report (A.R.F.)

The specimen, as received, was incised by parallel sections, and placed in Formol-methyl-alcohol; microscopical sections being stained with Haem-alum and Eosin and also by Giemsa.

The skin over the lesion was much reduced in thickness, the majority of the Malpighian papillae having disappeared. The roots of hairs and sweat glands had also largely disappeared in an exceedingly dense cell-infiltration, which penetrated the corium for some distance. This, on examination with higher powers, was found almost exclusively mononuclear in character, the majority of the cells being of small size. Paler areas of larger mononucleated elements, however, were present as rather definite nodules in the midst of the small-celled infiltration. These were found filled with enormous numbers of parasites, very few occurring outside the cells. They were not so plentiful immediately under the skin.

Case XI. Note by Dr. Bitter, October, 1908. The case was that of a native officer of the Egyptian Army in Cairo, who had never been out of Egypt. He had small disseminated tumours, elevated about two to three millimetres, which were not ulcerated and covered with dry, whitish-coloured scabs of epithelium. The diameter of the individual tumours varied from two to ten millimetres. About twenty of these tumours were found on the right forearm from the elbow down (Pl. XV, fig. 9), three on his face, one on the forehead and two in the angle of the nose. He had consulted Dr Scheuber, a dermatologist in Cairo, who first thought it might be a case of leprosy, and who sent the patient to me for bacteriological examination. The research for leprosy bacilli was, however, negative; but I found the Leishman-Wright bodies in smears as well as in sections of an excised tumour. The parasites were situated in big mononucular cells (macrophages) which were abundant in the stratum just underneath the epithelium.

The same dermatologist brought, a few months later, some slides and an excised tumour to Dr. Dreyer, telling him that they had been taken from a Roumanian lady who had lived in Cairo for several years, and who was suffering from a similar affection on the face and neck. The size of the tumour was, however, bigger. Her sister was said to suffer from the same affection. Dr. Dreyer found a good many Leishman-Wright bodies in cells of the same type as in the case of Dr. Bitter.

*Case XII. Note of a case by Captain M. F. White, I.M.S.** (Pl. XV, fig. 14). The papules were first noticed in February, 1909, in Bushire (Persian Gulf), where cases were scarce at the time, none of the Europeans being affected. Had been in Baghdad two months previously.

The 'flea-season' was on in February and not much notice was taken of the spots. They consisted of a group of five small spots on the dorsum of foot, and two others further up about an inch away were noticed later. They varied in diameter from 3 to 5 mm. They were slightly raised with a dull red periphery, with a small scab in the centre, raised in some, depressed in others. The whole area of the five spots was slightly raised and inflamed. They gave rise to no symptoms, pain, or trouble whatsoever, except that occasionally after a hot bath they gave rise to slight tingling and became slightly swollen and red. Otherwise they have shown no sign of increasing or decreasing, and at the present time, fourteen months after they were first noticed, they are practically in the original condition.

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* We beg to express here our thanks to Captain White for his note and photograph, and to the Authors for permission to include it in this paper.—EDS.

For the last month, two of the spots which have been used for microscopic examination, have taken to remaining open for a few days, but always heal up again with the aid of a simple dressing.

Smears were taken in April, 1910, and typical parasites (*Leishmania tropica*) found. None were found in the peripheral blood, though many attempts were made to find them.

Cultures were tried twice on sodium citrate, acidified with citric acid, and incubated at 22° C. according to Rogers' method, but no result was obtained. Cultivation was then tried on blood agar, with positive result in each tube inoculated. The cultures showed *Herpetomonas* forms after two days' incubation, but it was found impossible to maintain them and to make subcultures owing to the contaminating micrococci of the skin.

PATHOLOGICAL HISTOLOGY OF THE LESIONS

It may at once be said that the pathological features of the two types above described present an underlying identity. The differences between them, although very great, both as regards their naked eye and histological characters, are, we believe, explicable on the ground of accidental influences depending on situation, exposure to friction, bacterial invasion, etc. The essential identity of the two forms is indicated not only by the presence in each of indistinguishable parasites, but by the essentially similar nature of the sub-epithelial infiltration which both present.

Warty Form. So far, we have only observed the warty forms in exposed situations where the skin is normally of considerable thickness, and where such growths are liable (in the absence of any protective covering) not only to bacterial invasion, but also to proliferative changes consequent upon the maintenance of a chronic inflammatory condition from irritative influences of a diverse nature.

In a thin paraffin section including the elevated margin of the lesion, one sees a number of vertical epithelial columns which penetrate the dermis sometimes to a depth of 7 to 8 mm. (Pl. XIV, fig. 10).

Small, apparently isolated down-growths of epithelium, the cells of which have a normal arrangement, also occur in the deeper lying tissue. Structures resembling 'cell-nests' have been frequently observed both in the epithelial columns and in the deeper epithelial collections just mentioned. The 'prickle-cell' nature of the epithelial down-growths is, as a rule, very clearly seen, the intervals between the cells being rather greater than usual by reason of inter-cellular oedema. The stratum granulosum and the keratinous layer are usually considerably increased in thickness. These features of the epithelium are simply those which might be expected to result from any chronic lesion. The 'cell-nests,' for instance, have no greater significance than they possess in other chronic lesions such as simple warts, scrofuloderma, etc.

The dermis itself is the seat of an exceedingly dense and uniform cellular infiltration which is continuous beneath the entire extent of the lesion. This infiltration is composed of cells of various kinds. The superficial strata of the dermis in the central parts of the lesion frequently contain considerable numbers of polymorphonuclear leucocytes, as well as free nuclei and other cellular detritus, the result of inroads by pyogenic bacteria. More deeply, however, the cellular infiltration is composed almost exclusively of mononuclear elements of various kinds. A certain proportion of these are indistinguishable from lymphocytes. In the midst of this cellular zone, small ill-defined areas are observed composed of mononuclear cells of much larger size than the preceding class. Such areas, seen under a low power, appear rather paler than the dense small mononuclear infiltration surrounding them. The parasites are found in the largest numbers in the interior of the cells composing such areas, though by no means exclusively confined to them. The infiltrated areas just described are fairly vascular, considerable numbers of small vessels of capillary character being present.

Hair-follicles and sweat-glands, which are often of course encountered, share to some extent in the surrounding infiltration, but have not been observed to suffer any degenerative or destructive change.

Deeply, the infiltrated zone gradually merges into the normal areolar tissue proper to the site.

Flat Form. The skin here, in contrast to the preceding form, is thinned and atrophied, the glands of the skin sharing markedly in the atrophic process. The papillae of the Malpighian layer are represented by very short processes, or are at the centre of the patch, entirely absent.

A sharply defined zone of cellular infiltration, practically identical in all respects with that described as pertaining to the warty form, is present under the thinned epidermis. Such a growth, examined in section with a pocket-lens, closely resembles the subcutaneous nodules sometimes seen in cases of leucocythaemia. The parasites, which occur in the large mononuclear cells described above, were present in such large numbers in both cases of this kind which we have examined, that the sections appeared to be crowded with them.

The parasites agree in every respect with the descriptions of *Leishmania tropica*. They are identical in both the warty and flat forms of the lesion (Pl. XIV, figs. 11, 12, 13).

Our two examples of the flat form were entirely free from micro-organisms. In the warty forms, on the contrary, these were constantly present. These micro-organisms were of many different kinds, and as they clearly represented a secondary accidental infection, we have taken no pains to determine their nature. They were most frequent in the superficial layers, and were never found deeply in the centre of the growth.

The examination of a large number of specimens has shown that in those cases in which bacteria were most plentiful, parasites occurred in very scanty numbers or not at all. Our opinion is that, so far from there being anything of the nature of a symbiosis between the two, the appearance of bacteria involves the extinction of the parasite. The reason why we have failed to find the parasites in so many of the cases reported, is probably that this process of extinction had reached a point where exceedingly few, if any, parasites had survived. The specimen which contained the parasite in largest numbers was one which contained no bacteria. Whether the bacteria ever destroy the parasites sufficiently to bring about a natural cessation of the process we have no means of judging, but it seems quite possible.

The condition is therefore essentially one in which the subdermic tissues are invaded by *Leishmania tropica*. Almost certainly, the parasite, after its entrance has been effected, multiplies in enormous numbers. The large non-granular mononuclear cells are those which are primarily attracted to the site of infection, and they harbour the parasite in large numbers in their interior. Whether intracellular multiplication of the parasite occurs or not, we have no means of stating with certainty, but it would appear probable.

The skin over the site of infection may or may not show hypertrophic changes; in other words, the resulting lesion may be either a prominent centrally ulcerated papilloma, or a slightly elevated non-ulcerated patch. In either case, the essential nature of the underlying infiltration is the same. We have, unfortunately, not been able to make any inoculation or cultural experiments with the parasite.

The facts to which we have called attention may be thus summarised:—

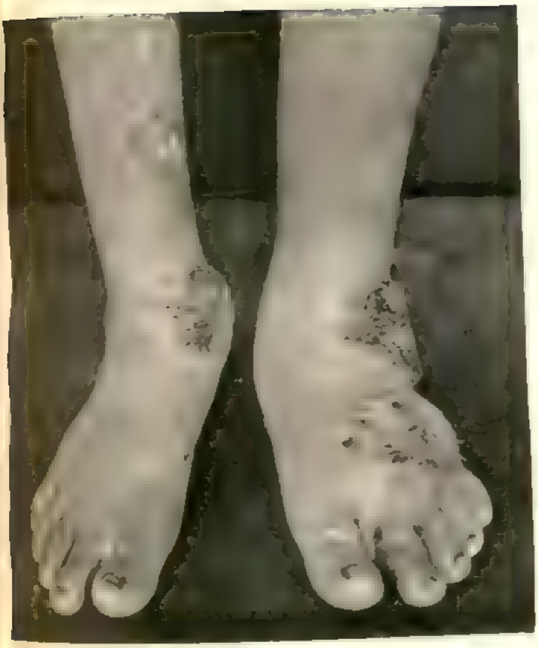
(1) Certain forms of skin affection caused by *Leishmania tropica* occur not infrequently in Egypt.

(2) They may be solitary or multiple, and in the latter case are almost certainly the result of auto-inoculation.

(3) They consist essentially of a mononuclear infiltration of the subcutaneous tissues which harbour, sometimes, large numbers of the parasites.

(4) The lesions manifest themselves clinically under two forms: the one, a slightly raised, smooth, flat patch; the other, a prominent warty growth. They run a chronic course, and are unaccompanied by constitutional disturbance.

(5) They are best treated by excision and immediate skin-grafting.



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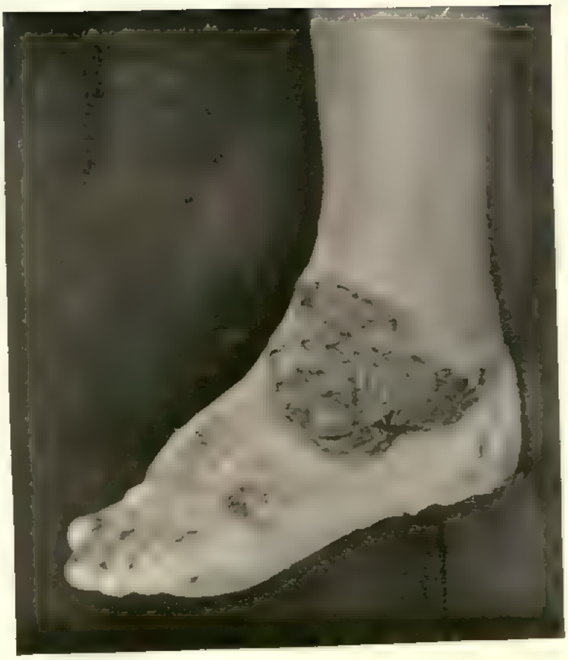

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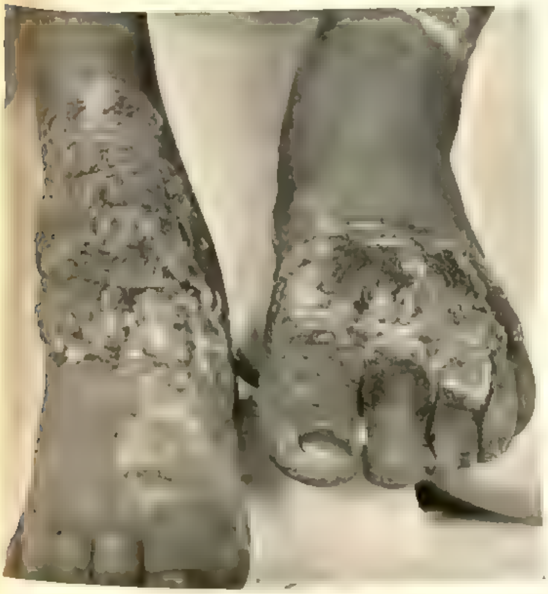

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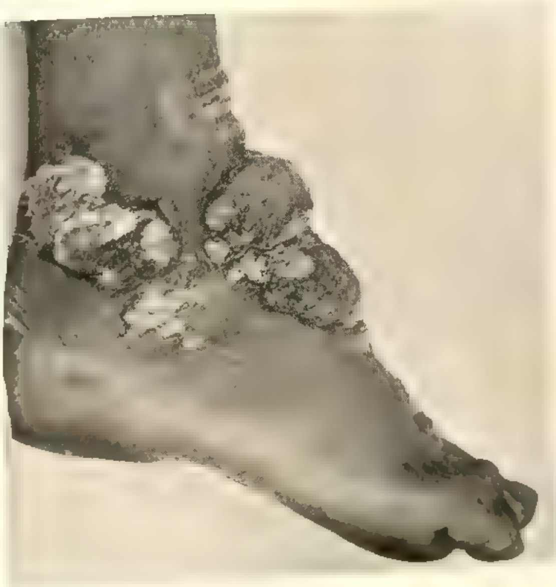

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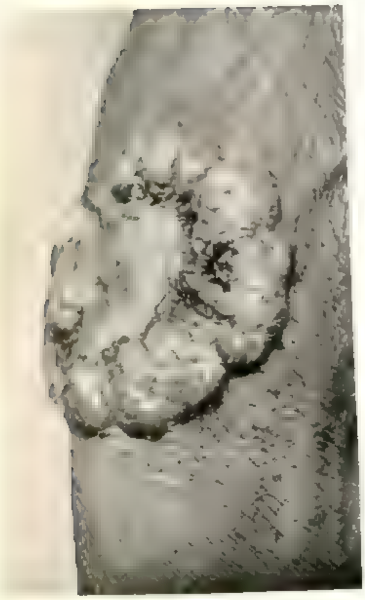
PLATE XIII.



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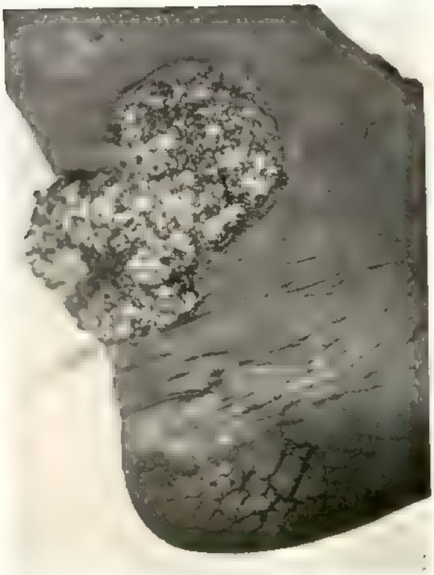

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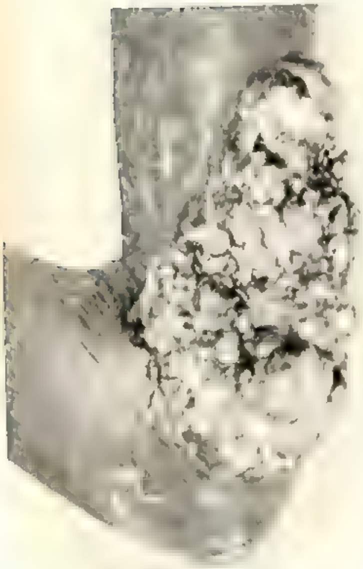

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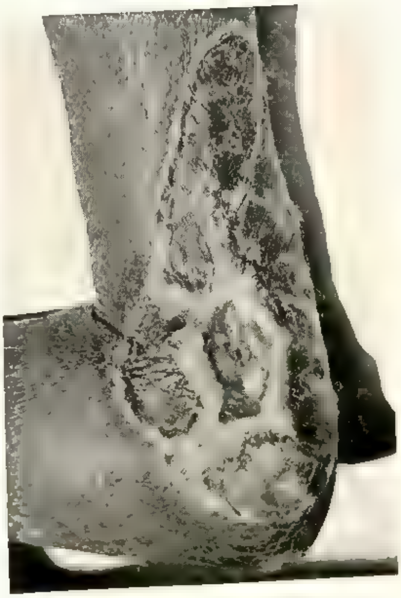

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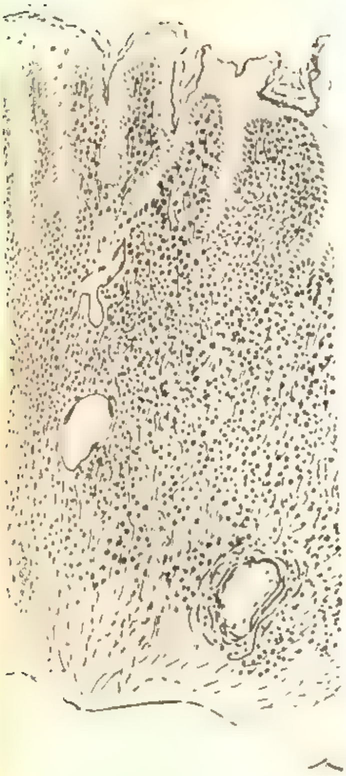

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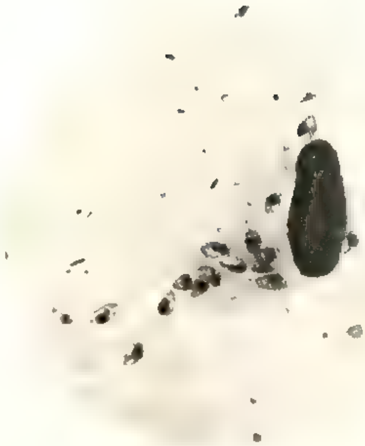

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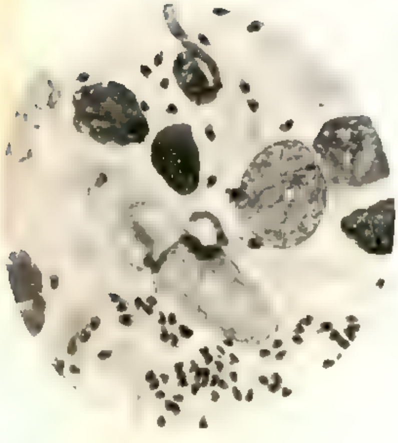

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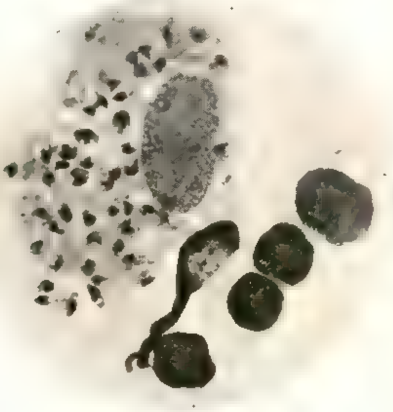

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CONTRIBUTION A L'ETUDE DU *POROCEPHALUS ARMILLATUS*

PAR
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ET
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(Received for publication 5 April, 1910)

Dans deux communications précédentes[1] parues dans ces *Annals*, Vol. I, No. 4, 1908, et Vol. II, No. 4, 1909, nous avons relaté des cas d'infection naturelle par *Porocephalus** chez l'homme, le singe, et certains grands serpents; ensuite nos expériences d'infestations expérimentale.

La présente notice à pour but de compléter certaines observations déjà signalées précédement.

I. INFESTATIONS EXPÉRIMENTALES DE L'HÔTE DÉFINITIF

On aurait pu objecter à nos expériences d'infestation expérimentales de l'hôte définitif, relatées dans ces *Annals*, Vol. II, page 311, que nos petits serpents, *Causus rhombeatus*, pouvaient être infectés naturellement. Cette Hypothèse nous paraissait inadmissible, car, malgré le grand nombre de ces *Causus* examinés par nous à Léopoldville, aucun n'avait été trouvé porteur de *Porocephalus*, analogue à ceux servant à nos expériences. Néanmoins nous avons repris ces essais d'infestation expérimentale, en essayant de suivre de plus près le sort des larves de Porocephalus chez le serpent en expérience; ensuite en donnant aux serpents un nombre déterminé de larves, à comparer au nombre de parasites trouvés ultérieurement à l'autopsie.

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* D'après une lettre privée que vient de nous envoyer le professeur Gedoelst, ces communications, ainsi que la présente, traitent en réalité de *P. armillatus* et non pas de *P. moniliformis*.

[1]

Le 25-IX-08. Nous tuons le singe No. 5 *Macacus*, infesté le 27-IV-08, c'est-à-dire, 5 mois auparavant, avec des œufs de *Porocephalus* du serpent No. 2 (*Python sebae* (Voir ces Annales, Vol. II, page 307).

Ce singe est fortement infesté par des larves enkystées, d'une façon analogue à celle décrite pour le singe No. 2 (ibid. pages 3 et 307).

Quelques unes de plus grosses larves de ce singe No. 5, servent à l'alimentation forcée de 3 petits serpents.

Serpent No. 1. *Causus rhombeatus*, reçoit le 25-IX-08 plusieurs larves. Celles-ci doivent être introduites au fond du pharynx pendant que l'animal est maintenu. Le nombre de larves introduites n'a pas été compté.

Le 28-IX-08 au matin ce serpent est trouvé mort. A l'autopsie nous trouvons des suffusions hémorrhagiques le long de la moitié antérieure du tube digestif, jusque vers la partie postérieure de l'estomac.

Dans la cavité stomacale, nous trouvons 2 larves sorties de leur membrane kystique, adhérant par les crochets à la paroi de l'estomac. 2 autres larves, non sorties de leur enveloppe et mortes, sont contenues dans la cavité stomacale. Dans le tissu conjonctif au niveau de l'estomac, mais en dehors de celui-ci, 2 larves libres.

Dans les sacs pulmonaires, 4 larves libres.

Dans l'intestin, nous ne trouvons aucune larve.

Il est peu probable croyons-nous, que la mort de ce serpent puisse être attribuée aux larves de *Porocephalus*. Il est plus admissible que la capture de l'animal et les manifestations violentes nécessaires pour l'introduction forcée des larves ont provoqué les lésions hémorrhagiques et la mort.

De cette expérience nous pouvons déduire qu'il faut aux larves de *Porocephalus* introduites dans le tube digestif du *Causus rhombeatus*, bein peu de temps pour en sortir et parvenir dans les sacs pulmonaires. Entre le moment de l'introduction des larves, chez ce *Causus*, le 25-IX-08 et l'autopsie le 28-IX-08, il s'est écoulé *moins de 3 jours.*

Serpent No. 2. *Bitis arietans*, reçoit le 25-IX-08, de la même façon que le serpent No. 1 et du même singe, 9 larves de *Porocephalus* enkystées.

Le serpent avait été quelque peu malmené pendant l'introduction des larves, mais paraissait avoir bien supporté les manipulations. Le 30-IX-08 nous lui donnons en nourriture un rat qu'il tue et avale, mais ensuite le serpent reste très indolent. Le lendemain 1-X-08, l'animal meurt, à peu près exactement 6 jours après l'infestation.

A l'autopsie, nous trouvons dans le tissu cellulaire au niveau de la bifurcation des bronches et du premier sac pulmonaire, des suffusions hémorrhagiques et une congestion violente de ce sac pulmonaire.

A ce niveau, dans le tissu conjonctif en dehors du sac pulmonaire, nous trouvons 1 Porocephale libre, un autre parasite libre dans le tissu cellulaire un peu en arrière du pylore. Dans le sac pulmonaire antérieur, il y a 1 parasite libre; dans le sac pulmonaire postérieur près de l'estomac 4 Porocephales libres, qui n'ont pas produit la moindre irritation. Le 9e parasite n'a pas été retrouvé.

Quand à la mort du serpent, nous devons faire les mêmes remarques que pour le serpent No. 1 *Causus rhombeatus*.

Entre le moment de l'infestation et celui de l'autopsie, il s'est écoulé 6 jours. Les parasites retrouvés chez le serpent No. 2, étaient déjà manifestement plus grands que ceux du serpent No. 1.

Serpent No. 3. *Causus rhombeatus*, est infesté le 25-IX-08, de la même façon que les 2 serpents précédents, au moyen de 5 larves enkystées du singe.

Ce serpent reste bien portant et est tué au bout de 2 mois, le 25-XI-08.

Les 5 Porocephales sont retrouvés dans les sacs pulmonaires, attachées aux parois par leurs crochets.

Il résulte de ces expériences: —

1° L'infestation des petits serpents mis en expérience est bien une infestation *expérimentale*, et non une infestation naturelle pré-existante.

En effet, chez le serpent No. 2, nous avons retrouvé 8 parasites des 9 introduits. La place occupée par l'un des parasites, dans la partie terminale du gros intestin, près l'anus, indique que dans certains cas, une partie des larves peut être éliminée par cette voie.

Cela a été le cas sans doute pour la 9e des larves introduites. Chez le serpent No. 3,—5 larves ont été données, et 5 parasites ont été retrouvés dans le sac pulmonaire.

En outre la place occupée par certains Porocephales dans le tissu cellulaire chez les serpents 1 et 2, indique suffisamment, croyons-nous, que ces parasites étaient d'introduction récente.

2° La migration des larves introduites chez le serpent par voie stomacale, se fait rapidement : chez le serpent No. 1, en moins de jours, des parasites sont parvenus dans le sac pulmonaire.

Ces nouveaux essais réussis d'infestation expérimentale de petits serpents par larves de *Porocephalus*, confirment ce que nous avons dit précédemment : *dans la nature les grands serpents s'infectent en avalant des animaux infestés de jeunes Porocephales pour lesquels ils constituent des hôtes définitifs.*

Nous avons recherché si un autre animal, par exemple, le singe, ne pouvait pas constituer un hôte définitif pour les *Porocephalus*, bien qu'après toutes nos observations, cette éventualité nous paraît peu probable.

Le 1-X-08, nous donnes per os à un singe *Macacus*, 5 *Porocephalus* provenant du serpent No. 2. Les parasites mis dans une capsule en gélatine sont introduits profondément dans l'arrière gorge de façon à éviter toute lésion ou déchirure par les dents.

Le singe reste bien portant. Tué le 10-XI-08, nous ne trouvons pas de traces des parasites introduits.

Le singe ne peut donc constituer un hôte définitif pour les *Porocephalus*.

II. INFESTATIONS EXPÉRIMENTALES DE L'HÔTE INTERMÉDIAIRE

A. L'homme. L'une de nos malades, *Gwangwate* qui avait ingéré de l'eau avec des œufs de *Porocephalus* du serpent No. 2 était encore en vie au moment de la publication de notre seconde notice (voir ces *Annals*, Vol II, pages 320 et 321). Cette femme arrivée à un stade incurable de la trypanose, avait été infectée par des œufs de *Porocephalus*, le 27-IV-08. Elle avait continué le traitement à l'atoxyl jusque peu de mois avant sa mort. Jamais cette femme n'a présenté le moindre symptôme morbide pouvant être attribué à la presence de *Porocephalus* dans ses organes, ou même permettant de soupçonner l'existence de ces parasites dans son organisme. La femme est morte de trypanose, le 11-XI-09, c'est-à-dire, 18½ mois après l'ingestion des œufs de *Porocephalus*.

Autopsie. Corps assez amaigri, pas de lésions cutanées.

Abdomen: pas d'exsudat dans la cavité péritonéale, pas de trace d'irritation. Nombreuses larves libres entre les anses, intestinales, dans les replis du mésentère, dans le petit bassin; elles sont mollement accrochées par leurs crochets. Nous recueillons en tout 73 larves libres.

D'autres larves sont encore enkystées dans le grand épiploon. Le foie renferme un nombre très considérable de larves enkystées, chaque incision faite dans l'organe, met à nu des larves enroulées et entourées de leur membrane kystique. Ni la rate ni les reins ne renferment de larves.

Cage Thoracique: pas d'exsudat dans les plèvres, pas de symptômes d'inflammation. Le poumon gauche renferme dans le lobe supérieur 2 larves enroulées et enkystées, le poumon droit, 1 seule larve.

Dans les *ganglions* lymphatiques du mésentère, nous retrouvons de rares larves assez petites, enkystées.

Pas de larves dans la paroi de l'intestin ni à l'extérieur de ce canal.

Les *dimensions* de ces larves sont relativement réduites: les plus grandes atteignent environ 22 mm. de longueur sur 2 mm. de largeur. Le plus grand nombre ne mesure que 15 à 16 mm. De cette analyse sommaire, deux points sont à retenir. D'àbord les dimensions relativement réduites des larves, malgré qu'il se soit écoulé depuis le moment de l'infestation jusqu'à la mort, le temps considérable de 18½ mois.

Nous devons en déduire que dans l'organisme humain, les larves de *Porocephalus*, se développent avec une lenteur extrême. Ensuite, l'absence de toute irritation, de toute réaction de la part des organes, constatée chez la femme Gwangwate comme chez les deux autres malades (ces *Annals*, Vol. II, pages 309-310), nous permet de conclure que les larves de *Porocephalus* ne provoquent guère d'irritation dans les corps humain.

B. Le Singe. Au moment de la publication de notre 2^e note (ces *Annals*, Vol. II, No. 4 le singe No. 4 (page 307) était encore en vie. Ce singe *Macacus* avait été infesté le 7-III-08 'avec des 'œufs de *Porocephalus* du serpent No. 1, gardés dans de la terre 'depuis le 7-XII-07, c'est-à-dire, depuis 3 mois. Les deux 'premiers mois la terre fut gardée humide, le troisième mois elle 'fut négligée, et resta plutôt sèche.'

Ce singe était resté constamment bien portant et très vif, lorsque le 5-II-1910, au matin, il est trouvé mort. Même la veille de sa mort, nous n'avions pas remarqué chez le singe des symptômes de maladie.

Autopsie. Dans la cavité abdominale, nous trouvons de l'exsudat sanguinolent assez abondant, et une inflammation aiguë du péritoine et de l'intestin. Nous y recueillons 100 larves vivantes et libres, réparties entre les anses intestinales, dans le petit bassin, ou accrochées à la paroi abdominale. Le grand épiploon renferme encore d'assez nombreuses larves enkystées.

Le foie présente une larve enroulée et enkystée à sa face antero-supérieure et une seule larve petite, enkystée dans le parenchyme de l'organe.

Ni la rate, ni les reins, ni l'intestin, ne renferment de larves

La cage thoracique est dans un état normal, les poumons ne renferment pas de larves.

A la face supérieure du diaphragme nous trouvons une seule larve enroulée et enkystée.

Les *dimensions* des larves libres recueillies dans la cavité abdominale sont très variables. La plus grande mesure 18 mm. en longueur, sur 2 mm. en largeur, la plus petite n'a que 8 mm. sur 1·5 mm.

Ce singe a succombé à une péritonite suraigue. Peut-elle être attribuée à la présence des larves *Porocephalus*?

Si l'on compare l'état macroscopique constaté chez nos autres singes infectés de *Porocephalus* soit naturellement soit expérimentalement, si l'on se rappelle l'absence complète de tout symptôme inflammatoire chez les 3 nègres infestés expérimentalement; l'on doit admettre avec nous qu'il est peu probable que la péritonite du singe No. 4 puisse être attribuée aux larves de *Porocephalus*.

Si elle pouvait avoir été provoquée par ces parasites, pourquoi ne s'est-elle pas produite plus tôt chez ce singe infesté depuis près de deux ans ?

Nous estimons que la péritonite du No. 4 peut être attribuée tout aussi bien à une cause fortuite, traumatique, par exemple. En effet, l'animal gardé à une chaine fixée autour de l'abdomen, a pu faire et faisait en réalité des mouvements violents. Une traction brusque, un choc, a pu produire un traumatisme abdominal, se terminant par la péritonite.

Quelle que soit la cause à laquelle l'on soit tenté d'attribuer la mort de l'animal, deux faits découlent de cette observation.

En premier lieu, c'est la ***longue vitalité des œufs de*** *Porocephalus.* Ce singe fut infesté comme nous l'avons dit plus haut avec des œufs de Porocephalus gardés pendant 3 mois dans de la terre, en somme dans de mauvaises conditions.

Ensuite nous ferons ressortir comme pour la femme Gwangwate, le développement extrêmement lent des larves de *Porocephalus* dans l'organisme de l'hôte intermédiaire-singe. Après 23 mois les plus grandes larves, rares, n'atteignaient que 18 mm., la moyenne 12 à 13 mm., les plus petites 8 mm. seulement.

Discussion Générale.

Des faits relatés dans cette notice comme dans les deux précédentes, nous pouvons déduire quelques faits intéressants pour l'histoire du *Porocephalus armillatus.*

Et tout d'abord, en ce qui concerne les hôtes intermédiaires, nous avons montré qu'en dehors de ceux signalés jusqu'à présent, homme, singe, chien, girafe, hyène, d'autres animaux encore, comme le chat, le rat, sont susceptibles d'infestation. Nous avons montré ensuite, par nos essais d'infestation expérimentale de l'homme du singe, du rat et du chat, que l'hôte intermédiaire s'infeste en avalant des œufs de *Porocephalus* adultes provenant des grands serpents.

Par l'infestation expérimentale du singe No. 4 moyen d'œufs gardés pendant 3 mois dans de la terre, en mauvaises conditions nous avons prouvé ***le grande résistance des embryons.***

Enfin, de nos observations, nous croyons pouvoir conclure que *l'hôte intermédiaire naturel*, du *Porocephalus armillatus*, est bien le singe. Nous avons signalé en effet (ces *Annals*, Vol. II, page 304), que sur 31 singes, nous en avions trouvé 9 infestés naturellement, soit 29%. Si l'on met en regard, d'un côté, la fréquence de l'infection *naturelle* chez le singe et sa rareté chez d'autres animaux ou l'homme, d'un autre côté, le fait que les grands serpents africains sont en effet mangeurs de singes, notre conclusion paraîtra logique. En ce qui concerne *l'hôte définitif*, nos observations n'ont fait que confirmer les connaissance acquises que les grands serpents africains *(Python sebae) (Bitis gabonica)* étaient les hôtes des formes adultes de *Porocephalus*. Chez le serpent, les parasites arrivent à développement complèt, ils s'y fécondent, ils y pondent leurs œufs.

Par nos essais d'infestation expérimentale de serpents plus petits *(Causus rhombeatus, Bitis arietans)*, nous avons prouvé que le serpent s'infecte par l'introduction par voie buccale de larves de *Porocephalus*.

Nos expériences ont prouvé ensuite qu'il faut aux larves bien peu de temps pour cheminer du tube digestif jusque dans les sacs pulmonaires. Chez le serpent No. 1, *Causus rhombeatus*, il a suffi de moins de trois jours.

Quand à la voie suivie par les larves de *Porocephalus* dans le corps du serpent, nous devons admettre qu'elles sont capables de *percer* la tunique stomacale, pour voyager dans le tissu cellulaire et pénétrer ensuite dans les sacs pulmonaires.

Nos observations ont mis en lumière la différence considérable dans la rapidité de développement des parasites chez l'hôte intermédiaire et l'hôte définitif.

Chez l'hôte intermédiaire ou accidentel (femme Gwangwate) ou naturel (par exemple singe No. 4), les larves, après un séjour respectivement de 18½ mois et de 23 mois, avaient à peine atteint 16 mm. et 22 mm. pour les formes les plus avancées. Par contre, chez l'hôte définitif, il a suffi d'un séjour de peu de jours, pour provoquer un développement marqué, appréciable à première vue.

Enfin, nos expériences et nos observations ont prouvé que les larves ou jeunes *Porocephalus* chez l'hôte intermédiaire soit naturel, ne provoquent guère de désordres. Parmi tous nos cas d'infestation expérimentale d'hôtes intermédiaires, seul un singe (No. 4) présenta, après 23 mois d'infection, des symptômes macroscopiques d'inflammation. Nous avons dit plus haut que nous ne pouvions pas sûrement les attribuer à la présence des parasites.

De même nous devons remettre en doute l'explication que nous avons donnée de la mort du soldat, chez lequel nous avons constaté en 1907, un cas d'infestation naturelle par larve de *Porocephalus* (ces *Annals*, Vol. I, page 499).

Nous avions dit alors que la larve devait avoir été enkystée dans la rate, qu'elle avait à un moment donné dù rompre le kyste et provoquer des lésions dans la rate, qu'elle était sortie ensuite de l'organe pour se loger dans la cavité péritonéale. Bref, nous avions cru alors à une fente purulente de la rate, suivie de péritonite, maladie dont la cause primordiale aurait été la larve de *Porocephalus*.

Après toutes nos observations et nos expériences d'infestation cette explication ne peut être maintenue. Ni chez l'homme, ni chez l'animal infesté expérimentalement, nous n'avons trouvé de larves à l'intérieur de la rate, alors que d'autres organes abdominaux, notamment le foie en contenaient.

L'absence de toute irritation et inflammation chez les singes infestés naturellement, et chez l'homme et les animaux infestés expérimentalement (à part le seul singe No. 4), prouve à suffisance que la mort du soldat doit être attribué à une autre cause qu'à l'action de la larve de *Porocephalus*. Cet homme doit avoir eu une affection de la rate ayant déterminé la fente purulente de cet organe, et cette lésion s'est compliquée d'une péritonite aigue.

CONCLUSIONS

L'hôte définitif de *Porocephalus armillatus* sont les grands serpents, *Python sebae*, *Bitis gabonica*.

L'hôte intermédiaire naturel est le singe.

L'hôte intermédiaire accidentel peut être tout autre animal ou même l'homme.

L'hôte intermédiaire s'infecte en avalant des œufs de

Porocephalus éliminés par les grands serpents, les œufs renfermant un embryon offrent une grande résistance.

L'hôte définitif s'infeste en avalant un hôte intermédiaire naturel infesté.

Chez l'hôte intermédiaire, accidentel ou naturel, les larves de *Porocephalus* ont un développement très lent le développement chez l'hôte définitif est rapide

Les *Porocephalus* introduits dans l'estomac de l'hôte définitif percent la tunique stomacale et arrivent dans les sacs pulmonaires par migration à travers le tissu cellulaire.

Chez l'hôte intermédiaire, les larves de *Porocephalus* ne provoquent de lésions anatomiques ou inflammatoires que dans des circonstances exceptionnelles.

ON THE ABSENCE OF A VESICANT IN THE ETHER EXTRACT OBTAINABLE FROM MOSQUITOS

BY

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The bites of mosquitos are well known to cause a more or less troublesome degree of irritation of the skin, which commences at the end of a few hours, and lasts from one to three days or more. The skin in the immediate neighbourhood of the bite becomes reddened, itchy and slightly swollen. If rubbed a wheal is generally readily produced. The degree of irritation varies with different individuals. Those who have lived long in mosquito-infested districts not unfrequently describe themselves as having become quite or nearly immune to mosquito bites.

The cause of this irritative effect of the bite of the mosquito is presumably to be found in the fluid injected by it into the skin when sucking blood.* The nature of the effective constituent of this fluid has not yet been determined and, owing to the small size of the mosquito, investigation is attended with difficulty.

The experiments which form the subject of this paper were made with a view of ascertaining if any substance possessing irritating properties when applied externally to human skin was present in the extract obtainable from mosquitos with the aid of ether. It is well known that in the Spanish Fly (*Lytta vesicatoria*) a strong vesicant, cantharidin, is present in amount, equal, it may be, to as much as 2 per cent. of the dried insect. Although the present investigation failed to reveal the existence of any irritant in mosquitos, similar to that present in *Lytta vesicatoria*, it may be of interest to any who contemplate working on similar lines to give the method employed.

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* F. Schaudinn (Generations und Wirtswechsel bei Trypanosoma und Spirochaete. Arb. aus dem Kais. Gesundheitsamte, 1904. Bd. 20, S. 419), finds that the contents of the oesophagus of the mosquito are effective in causing irritation of the skin probably by the agency of the fungi they contain. The salivary glands are not irritant.

The procedure followed was similar to that adopted in the extract of cantharidin from Spanish Fly. About 500 mosquitos, consisting of various species of Culicines and Anophelines (chiefly the former) were collected in Nyasaland (Upper Shire River). The weight of these, after drying at 110° C., was 0·478 gr. The dried mass thus obtained was ground in an agate mortar, and mixed with one-third of its weight of magnesia. Water was then added, and the mixture evaporated to dryness in a porcelain capsule on a water bath. Dilute sulphuric acid was then added until an alkaline reaction was no longer obtainable, after which the water present was again driven off on a water bath. The dried mass thus obtained was next extracted with ether in a Soxhlet apparatus. From the extract thus obtained ether was removed by distillation, and the residue kept at 100° C. until all water was driven off. The residue was then again dissolved in a small amount of ether, and applied drop by drop to an area of human skin (flexor surface of the wrist) about 15 mm. in diameter. A small amount of solid material, not taken up by ether, was further extracted with chloroform, which was then allowed to evaporate on the same area of skin.

No irritant effect was, however, produced, the skin remaining free from redness or itching. The area to which the ether extract had been applied was kept undisturbed for fourteen hours without any change manifesting itself.

It would appear, therefore, that the irritant action of mosquito bites cannot be attributed to the existence in these insects of any substance possessing a vesicant action.

FACTORS IN THE TRANSMISSION AND PREVENTION OF MALARIA IN THE PANAMA CANAL ZONE

BY

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INTRODUCTION

In every malarial region it is important that the species of mosquitos common to that region should be recognised, their breeding habits should be studied, and a determination made of the species of Anophelines, hospitable to malaria, and those transmitting it. The English observers, Stephens and Christophers, noticed that certain species of Anophelines were natural transmitters of malarial fever, while others were rarely, if ever, found infected naturally, although it was possible to infect them under laboratory conditions. We know that the breeding habits of Anophelines vary, too, considerably, and it may be said that there is as much selection of breeding places by Anophelines as there is selection of feeding grounds by fish. Trout, salmon, and bull-heads have their analogues among Anopheline larvae; some of the latter requiring fresh aerated water, or water containing much green algae. Others are found in tree-holes and recesses of epiphytic tree plants, such as Bromelias, where they prey upon other species, while others preferring fresh aerated water are so adaptable that they will flourish in sewage streams, or in brackish water containing half its volume of sea water.

Some species require an abundance of sunlight, while other sylvan species prefer shady pools in which chlorophyll-bearing algae are relatively absent. The Anophelines insusceptible to malaria may be more limited in their choice of breeding places, so that in the work of malarial mosquito destruction the latter may be disregarded, and attention given wholly to the breeding places o those species responsible for the transmission of malarial fever.

With regard to man as a host, it is necessary to have some knowledge of the limits of his infectiousness, i.e., the number of sexual forms of the malarial parasite necessary to infect susceptible mosquitos.

Besides the question of hospitable species of Anophelines, there are other matters of much importance, such as latent malaria; the effect of quinine on the parasites in man; the value of various larvacides; algacides; agents destructive to ditch grass, and a knowledge of the quality of wire screening, and the size of the mesh necessary to keep out mosquitos.

OUTLINE OF THE SUBJECTS CONSIDERED:

Anophelines of this region.
Collection of larvae.
Breeding out mosquitos and methods of feeding.
Biting—infecting experiments.
Estimation of gametes.
Care of mosquitos after feeding.
Method of examining for zygotes and sporozoites.
Description of the malarial parasite in the mosquito.
Table of infecting experiments.
Notes and conclusions from table of infecting experiments.
Limit of infectiousness of man.
Nōtes on the bionomics of Anophelines.
Effect of salt or sea water on larvae.
Experiments with larvacides.
Experiments with agents destructive to vegetation, grass and algae.
Experiments with screening of various mesh.
Relative value of wire screening of various composition, based on practical tests and chemical analyses.
Note on the value of the practice of killing Anophelines found in quarters and barracks.
Effect of quinine upon the parasite in mosquito and man.

The following is a list of Anophelines of the Canal Zone:—

Cellia argyrotarsis, R.D.
Cellia tarsimaculata, Goeldi
Cellia(?) gorgasi, D.K.
Cellia albimana, Wied.
Anopheles (?) cruzii, D.K.
Anopheles (?) apicimacula, D.K.
Anopheles (?) punctimaculata, D.K.
Arribalzagia (?) malefactor, D.K.
Anopheles (?) eiseni, Coquill
Anopheles francisanus, McCrack
Anopheles pseudopunctipennis, Theob.

The above eleven species of Anophelines have been collected in the Canal Zone during the past five years. They are not taken, nor do they exist in their breeding places, in anything like equal numbers. For example: Only one specimen of *Ce. (?) gorgasi* has been found. Of the eleven species, the commonest ones are *Ce. albimana A. pseudopunctipennis* and *Arr. (?) malefactor*, but this again must be qualified by stating that the predominance of a species varies from season to season and from place to place. In certain villages, upon going through the barracks only *Ce. albimana* will be found, while in other villages, from five to ten per cent. of the mosquitos will be *A. pseudopunctipennis*, and at Ancon during October, 1908, 27 per cent. were *A. malefactor* and 72 per cent. *Ce. albimana*. Mr. A. Busck, of the Bureau of Entomology, United States Department of Agriculture, who collected and made observations on Zone mosquitos during 1907, gave it as his opinion that *A. pseudopunctipennis* was the commonest Anopheline during the period of his stay.

The necessities of the canal operations in excavating and filling, change the topography of districts and localities so as sometimes to convert salt marshes into fresh water ponds, or to make tracts of land containing few Anophelines, into a vast swamp in which they luxuriate. On the other hand, swamps and breeding places may be drained or filled in the work of excavation. These factors, among others, influence the number and variety of species in a locality.

The commoner Anophelines of the Canal Zone may be divided into three groups:—

(A) The white hind-footed group comprising:
- *Ce. argyrotarsis*,
- *Ce. albimana*,
- *Ce. tarsimaculata*.*

(B) The leg uniformly coloured group comprising:
- *A. pseudopunctipennis*,
- *A. franciscanus*.

(C) The spotted leg group comprising:
- *Arr. (?) malefactor*,
- *A. (?) apicimacula*.

These groups present well-marked differences in the markings of adults, in the breeding habits and anatomical characters of the larvae, and, as will be shown, they possess varying susceptibilities to malaria.

The following are descriptions of the species of Anophelines of the Canal Zone:—

Cellia argyrotarsis

Thorax with mesonotum bluish grey, with three more or less longitudinal lines and with pale scales over the mesonotum, and sometimes traces of two dark lateral spots. The abodmen dark, dusky-brown, with a few creamy scales. Legs covered with dark scales, with some of the tarsi apically white banded; last three joints of hind legs pure white, and also apex of first; costa dark with two distinct and several smaller pale spots.

♀ Head black, with white upright spatulate scales in front, black behind and at the sides, a tuft of white hairs projecting forwards between the eyes. Eyes black; antennae dark, with pale silky pubescence and brown hair; basal joint dark, a few patches of white scales on the first few basal joints; palpi covered with long black scales, especially towards the base; apex pure white, and there are also two narrow white rings on the apical ends of the joints; ventrally, the penultimate joint has a number of yellowish-white scales, which sometimes seem to form almost a ring; proboscis clothed with short dark scales.

Thorax with a bluish grey sheen, with three more or less distinct longitudinal lines, the middle one most distinct, and of a purplish hue, with pale scales scattered over the mesonotum; scutellum dark towards the middle; mesonotum deep brown; pleurae dark, with here and there frosty tomentum (there are traces of two dark lateral spots on the mesonotum, which are clearly seen in the St. Lucia specimen).

Abdomen dusky purplish brown, clothed with creamy yellow scales, especially in the middle region of the segments; the segments have lateral tufts of grey scales on the posterior borders, projecting from the sides; hairs long, deep bright brown; viewed with a pocket-lens the abdomen is almost black in ground colour; in other specimens dull yellowish reflections may be seen.

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* According to Theobald (Monograph of Culicidae, Vol. V, p. 69, identical with *albimana*.—Ed.

Legs yellowish, covered with dark brown scales; first two tarsi of the forelegs apically white, last two joints dark brown, four midtarsi also with small pale apical bands; mid metatarsi and first two tarsal joints with minute apical yellow bands, last two indistinctly banded; in the hind legs the last three joints are pure snow white, and also the apex of the first; ungues very dark.

Wings with the costa dark, with four distinct and several smaller white patches; there are also numerous patches of dark scales, which vary to some extent, over the wing areas; in the ♀, from which this description is taken, the fourth long vein is covered with pale dusky scales, whilst in a ♀ from St. Lucia, it is creamy white; halteres with pale stem and fuscous knob. Length, 4 to 5 mm.

♂ Palpi dark brown, with scattered white scales, especially on the last swollen joints; hair-tuft pale; there is a pale ring at apex of the apical and base of the penultimate joint; antennae brown, with brown plumes; proboscis brown and narrow. The white scales on the head extend nearly over the neck; scales on the thorax white; the larger ungues of the fore-feet biserrated. Length, 4 to 5 mm.

During the period in which these experiments were being conducted I received very few specimens of this species, the sources being Miraflores, Ancon, Culebra, Paraiso and Corozal. Two specimens of *Ce. argyrotarsis* bit a patient having one crescent to 200 leucocytes and neither became infected. The patient was possibly an unfavourable case, and the experiment was not controlled by biting susceptible *Ce. albimana* at the same time. On December 2nd, from some Anophelines collected in labour cars at Corozal, one specimen of *Ce. argyrotarsis* was found containing a malarial zygote, 29μ in diameter, with fine discrete pigment.

Cellia tarsimaculata

This mosquito resembles *Ce. albimana* very closely, except for the different arrangement of the white bands on the palpi. This mosquito was found to transmit malaria.

Cellia albimana

This form resembles the type in all respects except that the last tarsal joint in the hind-legs has a very distinct and persistent deep black basal band. The thorax is rather browner in some specimens, and there are only two white bands to the ♀ palpi. The forelegs have dark-scaled femora, pale underneath, with a small white knee spot, the tibiae dusky-scaled and also the metatarsus above, pale below, apex white; the first two tarsi have yellow apical bands, the third dark, and the last clay coloured; mid legs with a large white spot near the apex of the femora; mid tarsi not definitely banded, but with a faint pale band sometimes at the apex of the metatarsus; the hind legs are dark brown, with the second, third, and apex of the first tarsal joints pure white, the last joint white, with a distinct black basal band; ungues as in the type. Wings much as in the type, but the pale scales are more yellow in colour. Length, ♂ 3·5 to 4·5 mm.; ♀ 4 to 4·5 mm.

This was the commonest species of Anopheline received as adults or larvae during the period embraced by this work, and was found to transmit both malignant tertian and simple tertian malaria.

Cellia (?) gorgasi

Palpi as long as the proboscis, mostly black scaled, the terminal and penultimate joints light scaled, except at the bases and apices; mesothorax grey with fine brown scales, a black spot in front of the scutellum, a pair of sublateral black spots medially; wings with the veins scaled in black and white, two very large black patches on the costa and a smaller one towards the base and a smaller one at the apex as in *Ce. albimana*, Weid. The rest of the wing is too much denuded to describe. Abdomen with groups of outstanding scales laterally at the apices of the segments, the dorsum clothed with yellow scales on a dark brown ground, the lateral tufts black. Legs mostly black-scaled, hind legs with the apical half of the second, the third, and the base of the fourth joints white-scaled, the remainder of the fourth and basal half of the fifth segments black, the third joint with a large black patch on the under side which reaches from near the base to beyond the middle. Length, 3·5 mm.

A single adult female of this species was collected by Mr. A. H. Jennings.

Anopheles pseudopunctipennis

Wings much as in *A. punctipennis*, Say, but the fringe with yellow spots. Legs, long, unbanded, brown, pale at the base. Fore ungues of ♂ unequal, mid and hind equal and simple.

♀ Antennae brown, basal joint testaceous, base of the second joint pale, and also a small pale band at the base of all the following joints: proboscis dark brown; labella yellowish; palpi dark brown, densely scaled at base; apex yellow, and also two narrow yellow bands below, slightly hairy, hairs black, except at the apex where they are yellow; clypeus dark brown. Thorax yellowish-brown (denuded), with a dark patch on each side of the mesonotum behind; metanotum deep brown; pleurae yellowish brown, with darker brown patches. Abdomen brown, the segment paler at the base, hairy. Legs deep brown; coxae, trochanters and base of femora pallid; knee spot pale; ungues equal and simple. Halteres with pale stem and fuscous knob.

Wings with two yellowish white spots on the upper costal border, rest of the edge black, rather densely scaled; first submarginal longer and narrower than the second posterior cell, its stem nearly as long as the cell; mid cross-vein a little nearer the base of the wing than the supernumerary cross-vein; posterior cross-vein still nearer the base of the wing; scales of the wings disposed as follows:—First long vein with three distinct large white spots, one at the base, one underneath a large costal spot, and one between; second long vein with a dark patch near its base, all the lower branch of the fork-cell dark, and most of the upper; third long vein mostly yellowish-white, with two black patches, one towards the base, and the other towards the tip; fourth long vein mostly pale, with two small black patches, branches of the fork cell all dark scaled; fifth long vein with a black spot near the base, rest mostly yellow, upper branch of the fork mostly dark, a small yellow spot at the apex and another towards its base, lower branch mostly yellowish, with a black apical spot; sixth vein with the basal half creamy, the apical half dark, except a small yellow patch where it joins the wing border; fringe brown, with yellow spot at the junction of each vein. Length, 5 mm.

♂ Last two joints of the palpi swollen and clavate, pale; basal joints dark brown, densely scaled with deep brown scales, with a narrow pale band not quite as long as the thin proboscis, which is brown with yellow labella; antennae grey, with narrow brown bands and flaxen brown hairs, the apical joint about half the length of the penultimate joint: basal lobe of the genitalia simple, claspers long and thin; fore ungues unequal, the larger one uniserrated, the smaller minute and simple; mid and hind ungues small, equal and simple. Wings much as in the ♀ but the fork-cells shorter. Length, 5 mm., with proboscis 7·5 mm. Habitat, Grenada (Dr. Hayton, per Dr. Daniel). Time of capture, February.

Observations—Very like *A. punctipennis*, Say, but can at once be told by the wing fringe being spotted at the apex of each nerve, and by the marking of the sixth long vein. The description is drawn up from two specimens in balsam, so that the scale structure is not evident. It is so very distinct, however, that it can easily be identified by the characters given below. I succeeded in infecting four specimens of this species.

Mr. August Busck found this species to be the commonest and most widely distributed one in the Zone during the season in which his collections were made, April-July, 1907.

Anopheles franciscanus

Male: Head dark brown, with short, dark, erect scales towards nape, emarginate and slightly forked, vertex and anterior part of occiput, with short, light brown scales not forked, a tuft of light brown hairs projecting forwards between the eyes, a row of similar hairs projecting forwards encircling the eyes posteriorly; eyes deep purplish brown; antennae about two-thirds length of palpi, yellowish-brown hairs, basal joint dark brown; palpi equalling proboscis in length with emarginate scales from base to tip on under and outer surfaces, those upon outer surface dark, upon under surface light, long light hairs covering distal third, becoming short and stout at the apex, a slightly banded appearance at base of three distal segments; two distal joints spathulate, proboscis scaled except labella, labella covered with medium stout setae, a few light hairs at apex.

Thorax: Prothoracic lobes dark; mesothorax dark brown at the sides, with scattered light hairs, a broad light-brown patch in the middle; within this light area a median line and obscure lateral lines; scutellum light with single horizontal row of hairs; metanotum dark without hair; halteres dark, covered with thick pubescence and emarginate scales; stalks light without scales.

Abdomen, basal area of each segment light, covered sparingly with long, light hairs; two stiff hairs on posterior margin of distal segment, stout hairs on margin of genital lobes.

Legs, coxae and trochanter light; trochanters, femora, tibiae, and tarsi covered with short, dark, emarginate scales and setae; ungues of front legs very unequal, the larger one with a large median tooth and a smaller basal lobe; middle ungues covered, with blunt basal lobes; posterior ungues equal, simple; posterior metatarsus slightly longer than tibia.

Wings with dark costa, with two distinct, nearly equal, yellow spots—one at distal end of subcostal vein, one at and involving distal end of first long vein; fringe dark, with a yellow spot at the end of each vein except at the end of the sixth; the first spot carried on to the first long vein, the apical spot carried past long vein on to the upper branch of the second long vein; the second long vein dark except for a few basal light scales; third long vein yellow in the middle, dark at the base and apex; light area at base of third long vein, carried over the fourth on to the upper branch of the fifth, with a few light scales at base; main branch of fifth long vein light, except at base and apex; distal half of sixth long vein dark, except at apex, basal half light; sub costal with a light spot carried to the first long vein (in one specimen the light spot on sub costal missing); third long vein prolonged slightly into the basal cell; first sub-marginal longer and slightly narrower than second posterior cell, stem twice the length of the cell; stem of second posterior cell prolonged to base of wing; supernumerary cross-vein adjacent to, or but very shortly removed from mid cross-vein and equal to it in length when removed nearer to apex of wing; posterior cross-vein a little longer than mid cross-vein and varying in distance from it, from one-half to almost twice its own length: third long vein prolonged slightly into the basal cell, darkest scales on costal, sub-costal and first long veins.

Palpi of the female equalling proboscis in length, light area at base of three distal segments, giving a banded appearance, clothed with scales, short hairs and setae, as in male, distal joints not spathulate; legs with the ungues equal, otherwise with the male.

No specimens of this species were infected, but as they are so close to *A. pseudopunctipennis* it might have been possible to infect a few if a large number had been used.

Arribalzagia (?) malefactor

♀ Palpi long, clothed with brown scales and black outstanding ones, which are grouped more or less in tufts, heaviest on the basal portion, a slight sprinkling of lighter scales among the brown ones, particularly at the bases of the dark tufts, occiput black scaled, the eyes margined with white above, and where they join it a tuft of white hairs; mesonotum grey with reddish and bluish tinge and small dark freckles tending to form longitudinal rows, sparsely distributed narrow yellowish scales, a spot at the base extending over the middle of the scutellum and two small sub-lateral back spots medially, all three of these show a lighter margin; abdomen slender, grey, with lateral tufts of outstanding black scales at the apices of the segments; legs with the femora and tibiae black, freckled with white; on the hind tibiae yellow scales predominate; tarsi black, ringed with yellowish-white, on the hind legs the first tarsal joint is dark at the base, light at the apex and has six white rings of different lengths, second joint narrowly white at base, broadly so at apex, with a moderately broad white ring near the middle and another narrower one between it and the base, third and fourth joints white ringed at base and apex with a broad central white ring, apical segment entirely whitish scaled; wing spotted, black and white, a large black patch margined with white on the costa near the middle, more basally a smaller costal patch and towards the apex another large one, all margined with white, scaling of the veins in patches of black and white scales, the third vein with a small black spot at the base, the sixth vein with many black dots and dashes. Length, 4·5 mm.

♂ Palpi with the apical portion clubbed, clothed with yellow scales with golden lustre, a narrow dark ring at the middle of the club, the shaft ringed with dull ochreous at the apex and at the constriction and broadly marked with the same colour on the apical portion; antennae pale brown and ferruginous with silky lustre. Length, 4·5 mm.

This large and beautiful Anopheline was received in good numbers from Miraflores and Ancon. Its name, however, appears to be a misnomer, for it could not be infected with malaria under the most favourable conditions.

Anopheles (?) apicimacula

As in *A. strigimacula*, D. and K., but with a distinct black costal apical spot on wing.

Anopheles (?) strigimacula

Proboscis black; palpi as long as the proboscis, black, a few whitish scales at the bases of the last two and middle of the long joint. Occiput black, clothed with erect black scales, a group of white ones in the centre of the vertex, a tuft of pale hairs at the vertex.

Mesonotum narrow, elongate, greyish, pruinose, a black spot below the lateral angle and one on the ant-scutellar space; vestiture of fine pale hairs arising from small black tubercles. Scutellum collar-like, greyish, with a black spot in the middle, clothed with pale bristles. Pleurae and coxae blackish with fine hairs, the coxae with patches of white scales.

Abdomen with the tip truncate, brownish black, clothed with numerous fine pale hairs; a row of lateral segmental posterior tufts of black spatulate outstanding scales; beneath with tufts of black scales and with scattered white ones.

Wings hyaline, the petiole of the second marginal cell as long as its cell; basal cross-vein distant about its own length from the anterior cross-vein; scales of the veins ovate, white on the costa and first vein, pale yellow on the others, with black scales and spots as follows :—

Three costal spots, the first small, involving two veins, the others large, involving three veins, the membrane beneath infuscated; no apical spot; costa and first vein with two or three little black spots between each of the large ones, the outer spot involving the base of the fork of the second vein; each fork with two little spots beyond; third vein with two spots at the base and two at the tip; fourth vein with a spot at the base, a large one involving the base of the fork, three on the upper branch and two on the lower; fifth vein with some black scales at the base, five spots on the upper fork, two on the lower; sixth vein with some irregular black scales toward the base, a spot in the middle and one at the tip.

Legs long and slender, black, speckled with white. Femora with about eight spots; tibiae with about fourteen, being about as many black scales as white ones; hind tarsi with ten spots on the first joint, second, third and fourth joints white at the base and tip, with a ring beyond the middle; fifth joint all white. Front tarsi with narrow white rings at bases and apices of the joints, the last entirely pale; mid tarsi not distinctly ringed. Claw simple. Length about 5 mm.; of the wing, 4 mm.

Anopheles (?) punctimaculata

As in *A. strigimacula,* D. and K., but the last vein with a row of black dots.

Only one specimen of this species is known. It was taken at Colon (W. M. Black, collector).

Anopheles (?) crusii

This species was examined only in the larval state, through the kindness of Mr. A. H. Jennings. No adult specimens were obtained and none ever received from or taken from quarters. This is significant on account of the peculiar tree-living habits of this species, and the probably groundless fear that it might be a malaria carrier.

Anopheles (?) eiseni

Near *A. maculipennis*, but with a patch of whitish scales on the first vein before its middle and another at its apex, also the apical fourth of the hind tibiae is yellowish-white. Halteres black, the stem whitish; coxae and a vitta on lower part of pleura, yellow, femora yellowish-brown, apical fourth of hind tibiae yellowish-white; antennae of male whitish, the first joint, last two, and fascia on each of the others, brown; scales of palpi black, those of apex and two bands in the female, three in the male, white; scales of occiput black, those in the middle of upper part white; mesonotum greyish pruinose, marked towards each side with a velvet black vitta; scales of abdomen black, the hairs yellowish, scales of femora and tibiae mixed black and whitish, those on the apical whitish portion of hind tibiae white, those on the tarsi black; tarsal claws on female simple; wings hyaline, the veins and scales brown, a dense patch of black ones at base of second vein, a larger one on the cross-veins and a smaller one at bases of first sub-marginal and of second posterior cell, a small patch of yellowish white scales on first vein before its middle and another at its apex, the latter spot encroaching upon the costal vein. Length, 3·5 mm.

It would have been of considerable interest to determine the susceptibility of *A. eiseni* and *A. cruzii* to malaria on account of their peculiar tree living habits, but it was almost impossible to obtain larvae of those species; and among hundreds of Anophelines caught within quarters and barracks which were examined and dissected, no specimens of this species were ever seen. It is extremely unlikely that they play any part whatever in the transmission of malarial fever in the Canal Zone at this time. The larvae of these species proved extremely interesting in comparison with the commoner species, such as *Ce. albimana*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, the anatomical characters of the former indicating definitely a very marked alteration in habits.

COLLECTION OF LARVAE

In order that a large number of adults could be kept on hand from day to day, it was arranged that sanitary inspectors at the various districts along the Panama Railroad should send bottles containing larvae and pupae to the laboratory daily. Special collections of larvae were also made and excursions to breeding places made from time to time. Upon receiving them at the laboratory, larvae were transferred to a glass moist jar partly filled with fresh water. Predaceous larvae, such as dragon-fly larvae were removed or killed and the Anopheline larvae transferred to feeding tanks containing algae and organic débris. These glass breeding tanks were placed on a table in front of a window having an eastern exposure so that they got direct sunlight for a few hours in the morning. The water in the breeding tanks was kept fresh and free from fouling by passing a jet of air through it with a Pacquelin cautery bulb, having a heavy glass perforated tip. This proved to be a very important addition to the technique of breeding out larvae. For shade and shelter a few *Lemma* plants were placed in the tank.

Several writers, collectors, and malarial investigators have mentioned the difficulties attendant upon the breeding out of Anopheles mosquitos from ova or very young larvae. Others have not mentioned the difficulties encountered or have not described the means used to obviate them. Banks, in the *Philippine Journal of Science*, Vol. II, No. 6, December, 1907, states, 'In laboratory 'breeding experiments the plants in the water begin to die within 'three to five days, while the larvae appeared to feed in a desultory 'manner. The time for their natural transformation to the pupae 'comes and goes and they still remain as larvae. The foulness of 'the water, due to organic decomposition, appears not to affect 'them, but on the other hand the lack of proper food seems to cause 'them to remain in an indefinite larval state until after three weeks 'or more they gradually begin to die.'

A very simple and satisfactory method for keeping the breeding tanks fresh and clean and free from decomposition was devised. The principle involved is that of aeration—preventing the development of an anaerobic condition in the water of the breeding tank by passing a fine jet of air through the water of the tank once or twice a day. It is well known, of course, that if natural water from streams, pools or ditches be placed in a glass tank, exposed to diffuse sunlight and undisturbed, after a few days the chlorophyll-bearing plants begin to disappear, a pellicle forms on the surface of the water, which contains bacteria, spirilla, flagellated protozoa, amoebae, etc. Beneath this surface film decomposition goes on rapidly, chlorophyll-bearing forms are destroyed or become encysted, and the various insect larvae and water-bugs die. The bacteria in the deeper portions of the water are largely anaerobes, and are associated with the putrefactive decomposition of the vegetable matter and animal matter in the tank. Banks describes exactly the effect on mosquito larvae of this putrefying vegetable matter. The odour arising from the tanks suggested the necessity of aeration to prevent the growth of the anaerobic bacteria. Acting on this suggestion an air jet was devised by attaching a thick glass rod, having a fine capillary central canal to the double bulb of the Pacquelin cautery apparatus and the breeding-out jars aerated for a minute or two morning and evening. The results were highly satisfactory, for the tanks were by this means kept clean and wholesome. The algae remained green and vigorous, the larvae active and developed rapidly into pupae, the water remained quite clear, in fact, with its floor of sand and clay and a few sprigs of green aquatic plant, such as *Lemna*, looked as tempting to drink as spring water. The temperature of the water in the tanks ranged between 72° and 84°.

BREEDING OUT—METHODS OF FEEDING

Pupae were collected in the morning and evening and placed in breeding-out tubes half filled with water and plugged with cotton. Each morning the newly emerged mosquitos were transferred to biting-jars. These biting-jars were modified from those described by Stephens and Christophers. After trying pickle jars and malted milk jars with much impatience, jars made of lantern chimneys were used. These were covered on both ends with crinoline gauze fastened with a string or a strong rubber band. Inside the jar was placed a circular ring platform of stiff paper, which many of the mosquitos used as a resting place. About twenty mosquitos would be placed in a jar over a small Stender dish* containing water on a Petri dish cover with a raisin or a piece of date for food. The jar would then be placed on a shelf in a dimly-lighted place, protected from ants by kerosene cups. Adult mosquitos may be kept alive for several days in such jars if they are fed with dates or raisins and a few drops of water, but if fed daily they will not bite and suck blood with alacrity. Anophelines do much better if they have one or two preliminary blood-meals before being fed with dates raisins or bananas. In several experiments it appeared that in the mid-gut of mosquitos gorged with stale banana and the associated bacteria and yeasts, the fermentative acid contents either destroyed the flagella of gametes, or in some other way prevented the development of zygotes in the mid-gut of susceptible mosquitos. In one experiment a patient whose blood contained almost as many crescents as leucocytes was bitten by four varieties of mosquitos; three of the four being susceptible species, yet none became infected. This failure might be attributed to the feeding on bananas.

Until the mosquitos were used for biting they were fed with dates, raisins and bananas, whenever they were to be kept for biting and dissections, it was found that those fed on dates and raisins gave much better dissections, while the mid-guts of banana-fed mosquitos frequently contained many yeasts and bacilli which caused the death of some of the mosquitos, apparently as a result of fermentation, and frequently yielded partly softened, tender and disintegrated intestinal tracts, which were removed intact with difficulty, and were occasionally lost. Bananas should never be used as food when dates or raisins can be obtained.

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* A 'Stender-dish' is one with a grooved lid fitting the rim of the dish.—EDS.

Mosquitos were kept in these biting-jars until a suitable case of malaria presented. The blood of patients selected to be bitten contained gametes or sexual forms of the malarial parasite in their circulating blood in numbers sufficient to infect susceptible anophelines. The method of determining this point will be considered later.

BITING AND INFECTING EXPERIMENTS

The earlier biting experiments were conducted at about 8 o'clock in the evening. After selecting a patient the jar of mosquitos was placed on the patient's forearm and covered with a heavy towel to prevent the disconcerting effect of light. Females several days old, that had been fed exclusively on dates or raisins, would generally bite greedily, and would feed on successive or alternate nights if given an opportunity. Most females would bite twenty-four or more hours after emerging, but before that period they would generally make no attempt to do so. When a jar is placed over a patient's forearm the mosquitos that are going to bite will almost always do so within a few seconds. If they show no inclination to visit the arm, a few gentle puffs into the opposite end of the jar, through the crinolin gauze, will make them change their position and frequently take up one on the patient's forearm. Another method that was used successfully was to have the patient's bed in the dark. If the jar were then gently tapped two or three times and aimed at a light down the ward some distance away the mosquitos would always take up a position on the gauze facing the light. The patient's forearm could then be carefully interposed and placed on the gauze, when the mosquitos would frequently take the hint and feed. Strong lights are powerfully attractive to female Anophelines and interfere considerably with successful bitings unless the jars are darkened by means of a thick cloth, the patients taken into a dark room, or the mosquitos very hungry.

Later, it was more convenient to conduct the biting about

4 o'clock in the afternoon, and on these occasions it was generally necessary to cover the jars well with a thick towel.

Two classes of patients were used: Spanish labourers and West Indian negroes, the former rarely objected to being bitten, the latter occasionally fretted a little. Both classes of patients were rather obtuse mentally and occasionally would not feel the bite, or would not be able to tell correctly the number of them. One reason for this is to be found in the limits of the number of separate points of pain appreciable within a radius of three inches—the biting area of the jar.

The Spanish patients' hands occasionally reeked with the odour of cigarette smoke, and a few of the West Indians had used citronella to prevent sand-flies from biting, but in neither instance did this interfere with the feeding of mosquitos. The hungry females, if undisturbed, will gorge themselves with blood and not infrequently will expel a drop or two of bloody fluid per anum. This not only occurs at the first feeding but has been observed at subsequent feedings.

After as many of the females as will bite have done so, the jars are taken to the laboratory, one of the gauze covers carefully removed and at the same time replaced by a card which is slipped out when the jar is placed on a Petri dish. The Petri dish contains a small Stender dish with a few drops of water and a split raisin or two. The jar is then placed on an ant-proof shelf in a dimly-lighted place, and under these conditions, with fresh food and water, Anophelines may be kept alive for two or three weeks. If one has fed mosquitos late in the evening and does not care to transfer or uncover them by artificial light, the gauze may be moistened with a few drops of tap water and the jars placed on the shelf until morning.

At the time of biting the patient, two or three good blood films for staining were taken and differential counts of leucocytes as well as the proportion of gametes to leucocytes made the following morning. By this means, and by an occasional leucocyte count, it was possible to estimate from films the number of gametes ingested by the mosquito in feeding.

ESTIMATION OF GAMETES

The estimation of the number of gametes ingested by the mosquito gives one an idea as to the grades of infection to be encountered in the mosquito, and this has indirectly a practical bearing which will be discussed later.

Leucocyte counts taken during convalescence in malarial fever generally show a number not far removed from the normal. It was possible, then, to estimate the number of gametes by counting the number met with while enumerating the leucocytes during the differential count. Such a gamete count was made from the blood of every patient bitten.

Mosquitos were weighed before biting and after biting, and the amount of blood ingested estimated in this way.

WEIGHTS OF MOSQUITOS

	Weight.
Ce. albimana bred in laboratory, twenty-four hours old, mid-gut empty	0·0008
Ce. albimana bred in laboratory, moderate blood feeding	0·0016
Ce. albimana caught in labour-cars, some blood in mid-gut, half developed ova	0·0019
Ce. albimana caught in labour-cars, much blood and early development of ova	0·0035
Ce. albimana caught at barracks, blood in mid-gut, no development of ova	0·0018
Ce. albimana caught at barracks, blood in mid-gut, no development of ova	0·0021

The average weight of mosquitos twenty-four hours old was 0·0008 gramme, while the average weight of mosquitos from the same brood that had bitten and taken a moderate amount of blood was 0·0016 gramme. The average weight of blood ingested being then 0·0008 gramme, approximately 0·001 gramme. Assuming the specific gravity of blood in malarial fever with slight anaemia to be 1·050, then 1·05 : 100 : : 0·0008 : 0·000761 = the volume of 0·0008 gramme of blood. Now if there were 22 gametes per 100 leucocytes, as in Experiment 38, and 6,500 leucocytes per mm.^{3} as they were, by actual count, there would be $22 \times 65 \times 0{\cdot}761 = 1088$ gametes ingested. If, under the most favourable circumstances, there are an equal number of male and female gametes, there should have been about 1,632 zygotes in this mosquito's mid-gut after three feedings, but as a matter of fact, there were only about fifty, showing a loss of about 97 per cent. This loss may be partly explained by an observation on the fate of gametes *in vitro* and *in vivo*, when it was noticed that fully 50 per cent. of gametes were phagocyted by polymorphonuclear leucocytes. This last is truly surprising, because this type of leucocyte in the circulating blood of man rarely plays any part in malarial phagocytosis, save in pernicious infections, when it will then engulf parasites and pigment. Usually it is the large mononuclear and endothelial cells which phagocyte malarial parasites and pigment.

In Experiment 41, blood specimens from a patient taken on admission contained 92 crescents per 100 leucocytes. Fresh preparations examined fifteen to twenty minutes after being taken, contained many pigmented extra-cellular parasites, some with quiescent pigment, others with pigment dancing in a circle around a granular centre. There were free flagella in some fields—two in one field. One flagellum was seen attached to several red blood cells and was moving with extreme violence, but without becoming detached. Quite a number of the gametes became phagocyted by polymorphonuclear leucocytes, one of the latter had phagocyted two gametes. Stained specimens showed numerous gametes within the polymorphonuclear leucocytes; one free, detached flagellum was seen. Some of the gametes were globular with linear pigment and a large chromatin dot, while others contained granular pigment, surrounding a chromatin ring, staining interruptedly and having an achromatic space on its interior and exterior.

CARE OF MOSQUITOS AFTER BITING

The mosquitos may be fed nightly or every other night from patients, or, if it is desired to ascertain the rate of development of zygotes, they are fed on dates after a single biting. The mosquitos should be kept in the biting jars with fresh food and very little water, just enough to favour oviposition and to keep the air within the jar moist, but not so much that they would be drowned.

It is very necessary that the infected mosquitos be protected from ants; otherwise valuable specimens that die during the night will be removed, with nothing but wings and legs to mark the loss.

Mosquitos may be transferred one at a time from the biting jar, and from day to day killed and dissected. Chloroform and cyanide may be used for killing. Chloroform is more conveniently used, but cyanide yields better preparations when it is desired to preserve most of the mosquito for identification, for the reason that cyanide causes the mosquitos to spread their wings.

METHOD OF EXAMINING FOR SPOROZOITES AND ZYGOTES

First it should be said that successful dissections can only be obtained with killed specimens. Mosquitos that have been dead twelve or more hours, particularly if they had fallen on the water, have become so macerated that the cells of the mid-gut or salivary glands separate and float away, it being impossible to retain the organ intact. When an examination of the salivary glands is desired, the wings and legs of the mosquito are trimmed off with a small, sharp entomological knife. The distal half of the thorax, with the abdomen, is removed by a transverse, clean cut, the mosquito being laid on a piece of white cardboard. With a small pair of forceps the proboscis is grasped, and the specimen laid on a drop of saline solution on a Stender dish cover under the dissecting microscope. The chitinous covering of the thorax, just behind the nape, is carefully slit, or torn, and the muscle organ beneath loosened slightly; then by pulling out the proboscis with one needle and holding the chitinous thoracic covering with the other, the salivary glands will be drawn out. They should be cut off from the head by a small, very sharp knife, and picked up with the point of a bright needle and placed in saline solution, 10 per cent. formalin, or films made for staining.

The sporozoites will be seen either free or in epithelial cells, or in the duct of the salivary glands; appearing as thin, slightly curved, spindle-shaped bodies, placed side by side, frequently as though matted together.

METHOD OF EXAMINING FOR ZYGOTES

The identified mosquito is laid on a piece of white cardboard, the abdomen cleanly and carefully removed by a transverse cut, just behind the thorax, and placed on a glass slide, or better, the under surface of a Stender dish cover, with a drop of saline solution. Under the dissecting microscope with the reflector properly adjusted, the hind-gut, mid-gut, Malpighian tubules and ovaries are withdrawn by pulling carefully on the last abdominal segment with one needle and holding the first abdominal segment by a corner with another needle. The mid-gut is separated from the hind-gut and Malpighian tubules as well as possible and transferred to a slide on the point of a needle which should be sharp and well burnished, where it may be examined in saline solution, formalin, or by other methods. When the mosquito has been dead several hours, the mid-gut cannot be withdrawn intact. In this event, it is generally necessary to split the chitinous abdomen open, and search carefully for as much of the mid-gut as may have held together. Zygotes can be detected with a low-power lens, Zeiss, 16 mm. objective and 8 and 12 oculars. If an absolute identification cannot be made at the time, all parts excepting the abdomen must be preserved intact, mounted with experiment number attached for final identification.

DESCRIPTION OF THE MALARIAL PARASITE IN THE MOSQUITO

As stated above, it was found that many gametes were phagocyted in the mosquitos' stomach (50 per cent. or more) by polymorphonuclear leucocytes. This materially diminishes the number of fertilised ookinets which reach and enter the wall of the mosquito's mid-gut. Notes from the following experiment throw some light on the changes occurring in crescents *in vitro*. The phagocytosis of crescents in the mid-gut was demonstrated separately, but films do not permit as careful study as those *in vitro*.

Experiment No. 20.—Blood contains, December 30, 67 crescents per 100 leucocytes. Temperature 97° continuously.

Differential Count of Leucocytes

Polynuclear	68	
Large mononuclear	4	
Small mononuclear	20	
Eosinophil	7	67 crescents per 100 leucocytes.
Mast	1	

In the fresh blood film, when it is drawn, numerous crescents are seen, but within five to ten minutes half the number of crescents have become vesicular and their pigment dancing; several have thrown out flagella, which are still attached after fifteen to twenty minutes. These vesicular gametes have become phagocyted by polymorphonuclear leucocytes; some of them at this period have parted with their flagella, because several free flagella were detected in the film. Stained preparations fixed immediately upon drawing the blood show nothing but crescentic forms, but films that have been kept moist from five to ten minutes before staining show that a very marked change has taken place in the crescents, many of them becoming either globular or irregular and distorted. One film fixed after ten minutes, and stained, showed nine crescentic forms, eight globular forms, with discrete pigment and several chromatin dots, some of which are certainly microgametes, and eleven irregular forms, as though becoming globular, or as crescents losing their stiff outline, and becoming flexible.

After fertilisation the microgametocyte becomes elongated (wandering vermicule, ookinet). Illustrations of wandering vermicules (after Grassi) are very much like the eleven irregular forms seen in the blood just described. These, then, may represent fertilised gametes.

The fertilised gamete, or ookinet, if it be not phagocyted, has abundant time to wander out of the blood clot and reach the gut wall, for the blood-meal of the mosquito is usually not expelled until after twenty-four hours.

The earliest form of the malignant tertian zygote was detected in the wall of the gut after the expulsion of the blood-meal, or after two and a half days. Satisfactory dissections and examinations cannot be made until the blood-meal has been expelled; consequently, after several trials, sixty hours after a feeding was the earliest period at which a search was made for zygotes.

In Experiment No. 204, a specimen of *Ce.* (?) *tarsimaculata* was killed sixty-five hours after a single feeding from a patient whose blood contained ten crescents per 100 leucocytes. Upon examination there were about fifty zygotes in the mid-gut. They were slightly oval in outline, with closely clumped quiescent pigment, and very little cytoplasm showing beyond the pigment, the diameter being about 5 μ. The zygotes become larger each day, though they d not always appear to grow at equal pace.

This variation in size may depend on location in the gut wall and its relation to nutrition. On the whole, however, the gradual increase in the size of the zygote is fairly uniform, as the following table shows:—

Experiment No.	Age of Zygote	Type of Parasite.	Size of Zygote in μ
16	4–4½ days	Simple tertian	12 × 16·5
16	4–4½ ,,	,, ,,	17 × 20
32	5 ,,	,, ,,	19·5
16	8½–9 ,,	,, ,,	48, 54
16	8½–9 ,,	,, ,,	48 × 54, 55·5 × 66 (Sporoblasts formed)
18	Sporozoites in salivary glands.	Smple tertian	
204	65 hours	Malignant tertian	5
9	60 ,,	,, ,,	9 × 11·25
41	2½ days	,, ,,	9 × 10·5
38	3 ,,	,, ,,	7 × 10
43	3 ,,	,, ,,	7 × 10
41	3½ ,,	,, ,,	12 × 15
38	4 ,,	,, ,,	12 × 15
33	3¾–4¾ ,,	,, ,,	12 × 13·5
42	4¾ ,,	,, ,,	21
36	5 ,,	,, ,,	21
11	6½ ,,	,, ,,	22, 28
38	7 ,,	,, ,,	45 × 57 (Contains sporoblasts)
13	7½ ,,	,, ,,	32 × 40
13	8½ ,,	,, ,,	39
36	11 ,,	,, ,,	Sporozoites in salivary glands
17	12½ ,,	,, ,,	30 (No sporozoites in salivary glands)

Measurements were made from fresh preparations in 10 per cent. formalin-saline under cover-slip with ocular micrometer (Zeiss).

The zygotes, as will be seen from the foregoing table, generally assume an ovoidal shape, one diameter being a little longer than the other. As they increase in size they become more vesicular, the periphery assumes a definite rim-like contour, and the pigment becomes more and more scattered, discrete, and ultimately, inconspicuous. During the first sixty hours the pigment is tightly clumped, but as the zygote becomes larger and vesicular, the pigment usually spreads out in lines or belts, sometimes in small clumps, or occasionally scattered and discrete. In Experiment No. 38 a specimen of *Ce. albimana* contained about fifty malignant tertian zygotes, three and a half days old (12 × 12 μ), in its mid-gut; the pigment was in the form of linear rods, and almost every zygote had a zonal arrangement of its pigment. The zone of pigment was made up of rods, end to end, each rod separated by a small space; sometimes the zonal pigment was concentric with the periphery of the zygote, sometimes at right-angles to the plane presented to the observer, but apparently always peripheral.

In Experiment No. 33, a specimen of *Ce. albimana* contained twelve to fifteen malignant tertian zygotes, three and three-quarter to four and three-quarter days old, 12 × 13·5 μ in size, mostly oval in outline. The pigment was bronze in colour, and in clumps, never in lines or belts. In experiment No. 36 a specimen of *Ce. albimana* contained malignant tertian zygotes five days old, 21 μ in diameter, with the pigment present in clumps. On counting the number of pigment rods in a zygote, and in crescents, it is evident that conjugation of gametes does not occur, for there is the same amount of pigment in each instance. In Experiment No. 42, a specimen of *Ce. albimana* contained several malignant tertian zygotes of three ages (three bitings from the same patient). Five or six zygotes were between one and a half and two and a half days old, and their pigment, of course, tightly clumped. One zygote, 21 μ in diameter, was globular and contained three clumps of pigment, while in several others of the same age the zygotes were oviform and the rods of pigment were arranged in pairs, scattered irregularly throughout the zygote, each one containing thirteen or fourteen rods of pigment.

In most of the zygotes, excepting the very young or very old forms, the pigment was arranged in lines or belts, but not uncommonly zygotes were seen of equal age in the same specimen, some with belts of pigment, and some having it in clumps.

The larger zygotes, containing sporoblasts, showed very little pigment. This is collected in one clump, usually near the periphery. The pigment is probably not destroyed nor extruded, but is partly obscured by the greater size of the zygote.

Some of the smallest zygotes contained dancing pigment, and in the larger zygote, with zones of linear pigment, the zone could be seen to change its position very slowly, but in these instances the pigment was not dancing.

When the zygotes reach the size of 39 × 45 μ, or 45 × 57 μ (seven to eight and a half days) a fine reticulum could be seen outlining the sporoblastic chamber; while others were dotted throughout with coarse round granules, the sporoblasts.

The capsule of the zygote ruptures about the eleventh day, the sporozoites making their way to the salivary glands, and leaving the collapsed, wrinkled, disc shaped envelopes behind in the outer layer of the mid-gut wall. The mechanism of the passage of the sporozoites into the salivary glands is not known, but these glands are more or less filled with sporozoites after the eleventh day.

The simple tertian zygote differs from the malignant tertian, first in the rapidity of its development, attaining a slightly greater size in a given time. In the younger zygotes the pigment is arranged in clumps or lines and slowly changes its position. The pigment may be clumped and dancing within vacuolated spaces of the zygote. In Experiment No. 16, tertian zygotes in *Ce. albimana* are 12 to 16·5 μ in diameter four to four and a half days after biting. The capsule of the zygote was well defined, but the zygote was apparently slightly larger than a malignant tertian zygote of the same age, and its cytoplasm was more coarsely granular than that of the malignant tertian parasite. The pigment is coarse and in clumps, not lines, and is not motile. In another specimen of *Ce. albimana*, in the same experiment, the pigment was arranged in two lines near the periphery of the zygote. Nine days after the first biting, one *Ce. albimana* with half mature ova, contained six to eight zygotes, full of faintly refractile, equally sized sporoblasts, 3 μ in diameter. These zygotes were 48 μ in diameter. Four or five zygotes were seen partly projecting from the outer wall of the gut. They were 48 by 54 μ and 58·5 by 66 μ. No pigment could be detected, but here and there faint clusters in groups, having a linear arrangement, indicating the development of sporozoites from sporoblasts. In this experiment the zygotes nearly reached maturity in nine days.

In Experiment No. 18, simple tertian sporozoites were found in only one of five acini of the salivary glands in a specimen of *Ce. albimana* eleven and a half days after the first biting. Four other acini were in plain view, but were entirely devoid of sporozoites. The sporozoites in the infected acinus were distributed in the lumen of the duct and in several of the distended vesicular epithelial cells, several hundred being present, their long axis being generally parallel with the duct.

In Experiment No. 36, malignant tertian sporozoites were found after the eleventh day in the red-stained hyaline salivary acini on both sides of the body in large numbers. Very few in the faintly stained, larger vacuolated acini, and none in the acini containing colloid globules; but the duct of the latter contained sporozoites, which were generally matted together.

Table showing data in relation to infected and non-infected mosquitos:—

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
2	1 M.*	?	5	3	0	No	Oct. 13	No. 9 18 B.	?
3	1 A.	E.A.†	10	3½	0	Yes	,, 19	47496	No
3	1 A.	E.A.	10	3½	5	Yes	,, 19	47496	Yes
3	1 A.	E.A.	10	4½	0	Yes	,, 19	47496	No
3	1 A.	E.A.	10	4½	0	Yes	, 19	47496	No
4	1 P.	E.A.	14 + −	5	5+ −	No	,, 27	48132	No
4	1 P.	E.A.	24 + −	5	31	No	,, 27	48132	No
5	1 M.	E.A.	3·5	4½	0	No	Nov. 10	48509	No
5	1 M	E.A.	3·5	4½	0	No	,, 10	48509	No
5	1 M	E.A.	3·5	4½	0	No	,, 10	48509	No
5	1 M.	E.A.	3·5	4½	0	No	,, 10	48509	No
6	1 M.	E·A.	16	3½	0	Yes	,, 11	48987	Yes
6	1 M.	E.A.	16	3½	0	Yes	,, 11	48987	No
6	1 M.	E.A.	16	3½	0	Yes	,, 11	48987	No
9	1 P.	E.A.	27	2½	0	No	,, 24	48987	No
9	1 P.	E.A.	27	2½	6 or 7	No	,, 24	48987	No
10	1 A.	E.A.	12	4 + −	Numerous	Yes	,, 27	48987	No
11	1 A.	E.A.	8	7	0	Yes	,, 30	48987	No
11	1 A.	E.A.	8	7	Present	Yes	,, 30	48987	No
11	1 A.	E.A.	8	7	168	Yes	,, 30	48987	No
12	1 P.	E.A.	[illegible]	2½	0	Yes	,, 30	48987	No
13	1 P.	E.A.	20	3½	0	Yes	Dec. 4	48987	No
13	1 P.	E.A.	20	3½	6 or 7	Yes	,, 4	48987	No
13	1 A.	E.A.	20	7½	3	Yes	,, 4	48987	Slightly
13	1 A.	E.A.	20	8½	Numerous	Yes	,, 4	48987	No
13	1 A.	E.A.	20	8½	Numerous	Yes	,, 4	48987	No
16	1 A.	Tert.	3+	4	6 or 8	Yes	,, 21	51147	No
16	1 A.	,,	3+	4½	20+ −	Yes	,, 21	51147	No
16	1 A.	,,	3+	8½	6 or 8	Yes	,, 21	51147	Yes
16	1 A.	,,	3+	8½	4 or 5	Yes	,, 21	51147	?
18	1 A.	,,	3	11½	1	Yes	,, 27	51431	Yes
32	1 A.	,,	17 + −	4¾	2	Yes	Jan. 25	53343	Yes
32	1 A.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
32	1 Ag.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	Partly
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	,,

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	O de
32	1 P.	Tert.	17 + —	4¾	o	Yes	Jan. 25	53343	Part.
32	1 P.	„	17 + —	4¾	o	Yes	„ 25	53343	Ye
33	1 A.	E.A.	11	4¾	12-15	Yes	„ 26	H.G.	Yes
33	1 A.	E.A.	11	4¾	12-15	Yes	„ 26	H.G	Ye
34	1 P.	E.A.	4	4½	o	No	„ 27	H.G.	N
35	1 Ag.	E.A.	8	2½	o	No	„ 28	53499	N
36	1 A.	E.A.	27	5	Present	Yes	„ 30	53499	Ye
36	1 P.	E.A.	27	5	o	Yes	„ 30	53499	N.
36	1 P.	E.A.	27	6	o	Yes	„ 30	53499	Y
36	1 P.	E.A.	27	6	o	Yes	„ 30	53490	Yes
36	1 A.	E.A.	27	11	Present	Yes	„ 30	53499	Yes
36	1 A.	E.A.	27	13½	„	Yes	„ 30	53499	—
37	1 P.	E.A.	29	3	o	Yes	Feb. 1	53742	N.
37	1 P.	E.A.	29	3	o	Yes	„ 1	53742	N
37	1 P.	E.A.	29	3½	o	Yes	„ 1	53742	Yes
37	1 P.	E.A.	29	3½	o	Yes	„ 1	53742	Slight
37	1 P.	E.A.	29	3½	o	Yes	„ 1	53742	.
38	1 A.	E.A.	29	2¾	Present	Yes	„ 1	53742	N.
38	1 A.	E.A.	29	3½	Many	Yes	„ 1	53742	Sligh
38	1 A.	E.A.	29	6¾	Numerous	Yes	„ 1	53742	Ye
38	1 P.	E.A.	29	11½	o	Yes	„ 1	53742	N.
38	1 A.	E.A.	29	11½	Many	Yes	„ 1	53742	Sligh
41	1 A.	E.A.	92	3½	Numerous	Yes	„ 5	53937	Ye
41	1 A.	E.A.	92	8½	?	Yes	„ 5	53937	?
41	1 A.	E.A.	92	10½	—	Yes	„ 5	53837	—
44	1 P.	E.A.	3·5	1½	o	No	„ 14	53937	No
44	1 M.	E.A.	3·5	2¾	o	No	„ 14	53937	No
44	1 M.	E.A.	3·5	4½	o	No	„ 14	53937	Yes
44	1 M.	E.A.	3·5	4½	o	No	„ 14	53937	No
44	1 P.	E.A.	3·5	4½	o	No	„ 14	53937	Slightly
46	1 A.	E.A.	2	1½	o	Yes	„ 17	53937	No
47	1 P.	E.A.	½	3½	o	Yes	„ 19	53937	No
47	1 P.	E.A.	½	3½	o	Yes	„ 19	53937	No
47	1 A.	E.A.	½	3½	o	Yes	„ 19	53937	Slightly
47	1 A.	E.A.	½	3½	?	Yes	„ 19	53937	Yes
47	1 A.	E.A.	½	3½	No	Yes	„ 19	53937	Ye
47	1 A.	E.A.	½	3¾	3	Yes	„ 19	53937	No
47	1 A.	E.A.	½	3¾	4	Yes	„ 19	53937	No
47	1 P.	E.A.	½	3¾	o	Yes	„ 19	53937	N
47	1 M.	E.A.	½	3¾	o	Yes	„ 19	53937	No
47	1 A.	E.A.	½	3¾	o	Yes	„ 19	53937	Slightly
47	1 M.	E.A.	½	3¾	o	Yes	„ 19	53937	No
47	1 P.	E.A.	½	3¾	o	Yes	„ 19	53937	No
47	1 P.	E.A.	½	3¾	o	Yes	„ 19	53937	No
47	1 P.	E.A.	½	3¾	o	Yes	„ 19	53937	No
42	1 A.	E.A.	10	1½	o	Yes	„ 6	53742	No
42	1 A.	E.A.	10	1½	o	Yes	„ 6	53742	No
42	1 P.	E.A.	10	1½	o	Yes	„ 6	53742	Yes
42	1 A.	E.A.	10	3¾	Several	Yes	„ 6	53742	Yes
42	1 M.	E.A.	10	3¾	o	Yes	„ 6	53742	Yes
42	1 A.	E.A.	10	4½	Present	Yes	„ 6	53742	Yes
42	1 A.	E.A.	10	4½	„	Yes	„ 6	53742	Yes
42	1 A.	E.A.	10	5¾	Many	Yes	„ 6	53742	Yes
42	1 A.	E.A.	10	5¾	„	Yes	„ 6	53742	Yes
42	1 A.	E.A.	10	5¾	3 or 4	Yes	„ 6	53742	Yes

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
42	1 A.	E.A.	10	5¾	3 or 4	Yes	Feb. 6	53742	No
43	1 A.	E.A.	5	2¾	5	Yes	,, 8	53742	No
43	1 M.	E.A.	5	2¾	0	Yes	,, 8	53742	No
47	1 P.	E.A.	½	5½	0	Yes	,, 19	53937	No
47	1 M.	E.A.	½	5½	0	Yes	,, 19	53937	No
47	1 Ag.	E.A.	½	5½	0	Yes	,, 19	53937	No
47	1 Ag.	E.A.	½	5½	0	Yes	,, 19	53937	No
47	1 M.	E.A.	½	5½	0	Yes	,, 19	53937	No
202	1 P.	?	0?	4	0	No	Aug. 24	Bed 90	Yes
202	1 T.	?	0?	4	0	No	,, 24	Bed 90	Yes
204	1 A.	E.A.	5 + —	3	40	Yes	,, 28	63472	Yes
204	1 T.	E.A.	5 + —	4	Many	Yes	,, 28	63472	No
204	1 A.	E.A.	5 + —	9	Many	Yes	,, 28	63472	Yes
209	1 T.	E.A.	5 + —	2	20	Yes	Oct. 5	65343	Yes
209	1 T.	E.A.	5 + —	2	22	Yes	,, 5	65343	Yes

*A. = *albimana*
M. = *malefactor*
T. = *tarsimaculata*
P. = *pseudopunctipennis*
Ag. = *argyrotarsis*

†E.A. = malignant tertian
Tert. = simple tertian

NOTES AND CONCLUSIONS FROM THE FOREGOING TABLE

Species	Number	Infected	Percentage infected
Arr. (?) malefactor	17	0	0·0
A. pseudopunctipennis	31	4	12·9
Ce. albimana	48 + 2	34 + 2	70·2
**Ce. argyrotarsis*	4	0	0·0
Ce. tarsimaculata...	5	3	60·0

* A naturally-infected specimen of this species has been found in barracks.

Out of several hundred mosquitos used in the biting experiments 107 gave satisfactory dissections, or paraffin sections, and it was determined that:—

> 70·2 per cent. of *Ce. albimana* became infected;
> 60 per cent. of *Ce. (?) tarsimaculata* became infected; and
> 12·9 per cent. of *A. pseudopunctipennis* became infected;

while none of *Arr. (?) malefactor* became infected, although several were placed in jars with *Ce. albimana* and bit at the same time persons from whom the specimens of *Ce. albimana* became infected.

It is concluded from this series of experiments that *Ce. albimana*, the common white hind-footed mosquito—an extremely hardy, rapidly developing, adaptable mosquito—is the transmitter of malignant tertian and of simple tertian malarial fever in the Canal Zone at this time. Specimens of this species infected with simple tertian parasites became infective between nine and eleven and a half days after the first feeding. When infected by malignant tertian parasites, sporozoites appeared in the salivary glands as early as the eleventh day in some mosquitos, and later than twelve and a half days in others.

Ce. (?) tarsimaculata appears to be as susceptible to the malignant tertian parasite as *Ce. albimana*, and no doubt if a favourable opportunity had presented it would have been found that *Ce. (?) tarsimaculata* would have been equally susceptible to tertian malaria.

A. pseudopunctipennis is only slightly concerned in the transmission of malarial fever, if at all, not only from the fact that only four out of thirty-one mosquitos under the most favourable conditions became infected, but from the additional fact that relatively few specimens are taken in quarters at this time.

Arr. (?) malefactor is not concerned in the transmission of malarial fever in the Canal Zone at this time.

Out of forty one mosquitos containing malarial zygotes, seventeen showed development of ovaries or ova. A few of these, however, might have oviposited before examination.

Out of sixty negative bitings, i.e. where mosquitos of any of the species bit and failed to become infected, twenty showed development of ova ; and to take the known susceptible species, *Ce. albimana* and *Ce. (?) tarsimaculata*, out of thirteen *Ce. albimana* and *Ce. (?) tarsimaculata* which failed to become infected six showed development of ova.

It would, therefore, appear that fecundation and development of ova are not necessary for the development of zygotes.

Under the most apparently favourable circumstances one or two out of the lot of susceptible Anophelines will fail to become infected, and these, no doubt, possess an active immunity toward the malarial parasite.

LIMITS OF INFECTIOUSNESS OF MAN

During the progress of the experiments it was noticed that patients were discharged after their temperature had become normal and when their peripheral blood occasionally contained more than a sufficient number of gametes to infect susceptible mosquitos. In order that a recommendation might be made for the continued treatment of these persons, it was necessary to determine, if possible, the limits of infectiousness of such individuals.

Several experiments were carried out, in which *Ce. albimana* bit patients whose gametes per leucocytes had been determined. These mosquitos were given but one blood feeding and fed subsequently on dates and raisins and then dissected. The limits were determined as being near one gamete per 500 leucocytes, or twelve gametes per mm.^{3}; but it must be understood that several factors are concerned in infections, such as number and phagocytic power of leucocytes; immunity of mosquitos, racial and individual; probable reaction of gut contents, as acid bacterial products or those from yeasts, may be inimical to the gametes; and again proportion of ♀ to ♂ gametes plays some part, besides the number of gametes ingested. In Experiments 20 and 28 patients' blood was rich in crescents which flagellated *in vitro*, and in the mosquito's stomach, yet mosquitos never could be infected from the patients.

Persons with more than twelve gametes per mm.^{3} must be regarded as gamete carriers, and, of course, should not be discharged from hospital nor should treatment be discontinued until gametes have been reduced well below the limits of infectiousness. This destruction and prevention of the development of the sexual forms of the parasite in man is a matter generally overlooked, but is of the greatest importance in delimiting malaria, and it may be accomplished by appropriate quinine treatment of all gamete carriers; by quinine treatment to destroy latent malaria, and by periodical blood examination of labourers and others in quarters where there is a high malarial rate. For the detection of gamete carriers and latent malaria, in order to carry out appropriate treatment, 30 grains of quinine sulphate in solution daily is an efficient dosage for the purpose required.

Experiment No. 47.—February 19, 1909, 8 p.m., jar containing *Ce. albima* *A. pseudopunctipennis* and *Arr. (?) malefactor* was applied to No. 53,637, who wa receiving 30 grains of quinine daily when his blood contained one crescent t 2 leucocytes = approximately + 30 gametes per mm.^{3}. The mosquitos were gi but one blood feeding and subsequently fed on raisins and dates.

Upon dissection on February 23-5 :—

9 *A. pseudopunctipennis* were not infected
4 *Arr. (?) malefactor* „ „
2 *Ce. argyrotarsis* „ „
3 *Ce. albimana* „ „ while
1 *Ce. albimana* contained three zygotes, and
1 *Ce. albimana* contained four zygotes.

Experiment No. 31.—January 25, 1909, a jar containing *A. pseudopun* *pennis*, *Ce. argyrotarsis* and *Ce. albimana* were fed from 53343, tertian malar fever, whose blood contained four gametes per 100 leucocytes.

Upon dissection, January 30 :—

3. *A. pseudopunctipennis* were not infected;
1 *Ce. argyrotarsis* was not infected;
1 *Ce. albimana* was not infected; while
1 *Ce. albimana* contained two zygotes.

NOTES ON THE BIONOMICS OF SOME OF THE ANOPHELINES STUDIED

The period of incubation of the ova of *Ce. albimana*, *A. pseudopunctipennis* and *Arr. (?) malefactor* was estimated as about thirty-six hours under the laboratory conditions, the temperature of the air and water ranging between 78° and 82° daily, an eastern window exposure with direct sunlight for three or four hours in the morning, and diffused sunlight the remainder of the day. The eggs are laid in the geometrical patterns usually seen, and at first are creamy-white colour, becoming in a few hours quite black, with white lateral air chambers.

The larval period varies with the species, food, efficient temperature, sunlight and environment; for example, ova of *Arr. (?) malefactor* and *Ce. albimana* of about the same age were exposed in the same breeding tank to an identical environment, food, water, air, and sunlight; but when *Ce. albimana* had pupated the larvae of *Arr. (?) malefactor* were only half grown. In one experiment *Ce. albimana* larvae pupated within twelve days, while *Arr. (?) malefactor* required sixteen to twenty days. Subsequent examinations of water from *Arr. (?) malefactor* pools and from the intestinal tracts of *malefactor* larvae indicate that the latter prefer shady pools in which chlorophyll-bearing algae, the chief food of *Ce. albimana* larvae, are relatively absent.

Ova of *Ce. albimana*, still creamy-white in colour, were placed in a breeding tank exposed to the morning sun on December 3; temperature of the water, 28·5 to 30° C. (78° to 82° F.). Of these ova five became larvae and pupated December 14 15. These pupae became imagines during the night of 16 17, making the period from ovum to imago about thirteen and a half days. Under these conditions they did not get as much sunlight as they would have received outside. Sunlight and the abundance of algae undoubtedly play a great part in the duration of the period of incubation. It should be added that these five mosquitos, two males and three females, were placed in a biting jar the morning they emerged—December 17, and that same evening each one of the three females bit and drew blood at once when applied to the arm of a patient. In this instance mosquitos bit when not more than twenty-hour hours old.

Into the same tank were placed sixty-eight larvae of *Arr. (?) malefactor* from ova laid December 3; one-third of the ova hatched the morning of December 5, the approximate age of the ova, or the period of incubation, was thirty-six hours. The tank was supplied with algae, spirogyra, and the water aerated. It was noticed very soon that the *malefactor* larvae did not mature as rapidly as the *albimana* larvae did; when specimens of the latter were full grown the *malefactor* were only one-third or half grown, and the *malefactor* larvae did not pupate until sixteen to twenty days after hatching.

HARDINESS OF *CE. ALBIMANA*

This mosquito is well fitted for the purpose of transmitting malarial fever. It is the commonest species here at the present time, outnumbering all others, excepting, possibly, *A. pseudopunctipennis*, which latter species is not very hospitable to the malarial parasite.

It breeds in a great variety of locations; besides the customary pools and margins of streams, collections of rainwater, during the dry season it may be found in the stinking water of sewage streams, brackish marshes, running streams, meadows, muddy pools, old crab holes, and in shady *malefactor* pools and river margins.

It matures more rapidly than either *A. pseudopunctipennis* or *Arr. (?) malefactor*.

It outlives *pseudopunctipennis* and *malefactor*, in confinement at any rate, which is a proof of its ability to persist, for when these species are placed in one breeding jar *malefactor* dies quickly, next follow *pseudopunctipennis*, while specimens of *albimana* survive for days longer.

DURATION OF LIFE OF MALES AND FEMALES IN CAPTIVITY

Male specimens of *A. pseudopunctipennis* which have been kept in breeding jars with females, and supplied with raisins, dates and water, have lived for eighteen days. Male specimens of *Ce. albimana* have lived twelve and a half days. On the other hand, a virgin specimen of *Stegomyia calopus* has lived for one hundred and ten days.

When virgin Anophelines have been given one or two blood meals two or three days after emerging they have lived as long as sixteen days. This is in rather striking contrast with *Stegomyia calopus*, which under similar conditions lives months to the Anophelines weeks. Several specimens of *S. calopus* virgins still under observation have lived for several weeks, and have oviposited as late as sixty days after emerging, though never in contact with males.

MUSICAL NOTE OF MOSQUITOS

The characteristic musical note of Anophelines is caused by the vibration of the proboscis, as the following observation indicates:—

A specimen of *Arr. (?) malefactor* was badly wet and sprawled; upon placing her upon a piece of filter paper and touching or approaching her proboscis the latter vibrated visibly, and emitted the characteristic high-pitched note; the wings were at rest, being stuck to the paper. This was verified again and again. Later I picked up a slightly water-sprawled, infected mosquito for dissection and held it over a few drops of chloroform; both wings were seen to vibrate rapidly as in flight, but noiselessly, while holding the mosquito by the last abdominal segment and touching one wing at its tip the opposite wing would immediately stop vibrating. Upon releasing the wing, both would vibrate noiselessly as before. The noise of the mosquito is due, then, to the vibration of its proboscis, and the wing vibration is dependently and automatically co-ordinated.

RELATIVE VALUE OF DIFFERENT FOODS FOR ANOPHELINES

After trial with bananas, I found that raisins and dates with water furnished the best food for Anophelines in confinement. A greater number of males and females may be kept alive during the few days after emergence if fed with fresh bananas, raisins or dates; but females fed on this diet daily would not bite with alacrity. Mosquitos fed daily or on alternate days on human blood made better dissections after the digestion and evacuation of their meal than those fed on bananas. The former would be fairly free from yeast and bacteria, and the mid gut and appendages would not disintegrate so rapidly after death. If the mosquitos were fed alternately on bananas and blood they would frequently die with an undigested hard mass of blood in the mid-gut, which must have been either impossible to digest or evacuate. The best method of feeding infected mosquitos would seem to be to feed two or three times on a patient favourable for infection, and subsequently with raisins, dates and water. Then, too, the acid contents of the mid-gut after banana feeding, with its fermentation, may interfere with the infection of mosquitos by malaria. In Experiments Nos. 20 to 28 a patient having at times sixty seven crescents per one hundred leucocytes in his peripheral blood was bitten by four varieties of mosquitos, *Ce. albimana*, *Ce. argyrotarsis*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, over a period of thirty-five days. During the intervals between biting, however, the mosquitos were fed on bananas, and none became infected.

IDENTIFICATION OF LARVAE

It must be evident that identification of Anopheline larvae in the field is of considerable importance, and in this region the malaria-transmitting Anophelines can be readily identified by certain anatomical characters. I have made no attempt to determine in detail all the anatomical characteristics of Anopheline larvae of this region; that has been done for some species by Knab. The chief anatomical differentiating larval characters of the common Anophelines of this region are these:—

Ce. albimana or white-footed group	Palmate hairs on all abdominal segments and sometimes on postero-external angle of thorax	Antennae without a tuft of hairs
A. pseudopunctipennis group	Palmate hairs on third, fourth, fifth, sixth, seventh abdominal segments, but none on the first and second. On the latter two, however, there is a rudimentary stalked tuft.	Antennae without a tuft of hairs.
Arr.(?) malefactor, or spotted-legged group	No palmate hairs on first and second abdominal segments, but palmate hairs on all remaining segments.	Antennae with a tuft of hairs.

These characters are very striking and sharply separate the groups, thus separating the malaria-transmitting *Ce. albimana* group from other varieties. With care it is frequently possible, even in muddy water, from an examination of the indentations of the surface film caused by the palmate hairs, to at once determine the presence or absence of members of the *albimana* group. In the latter group there is no break in the indented film, but in the two former groups there is a well defined non-indented break in the film, due to the lack of palmate hairs on the first and second abdominal segments.

The size of the palmate hairs on the postero-lateral angle of the thorax and the presence of these hairs on the thorax, first and second abdominal segments, is subject to some variation. It would seem that the white hind-footed group are undergoing some variation with regard to the size and location of these hairs and apparently they are becoming rudimentary or vestigial on the thorax and first abdominal segment.

FOOD OF ANOPHELINE LARVAE

The generally separate and distinct breeding places of *Arr. (?) malefactor* and *Ce. albimana*, for instance, naturally suggest that their food might also be different. Dissection of specimens of *A. pseudopunctipennis* and *Ce. albimana* in all instances discloses much green algae in the intestinal tract, while specimens of *malefactor* usually contains much dark brown, unidentified vegetable fibres, brown organic débris, and conidia resembling those of *Pestalozia trunculata*, *Leptosporum bifurcatum*, *Paramoecia*, etc., *Rotifer vulgaris*. This indicates that *Ce. albimana* and *A. pseudopunctipennis* prefer sunny pools, while *Arr. (?) malefactor* prefers shady ones, where there is a relative absence of chorophyll-bearing forms. This is not intended as an absolute statement, because during the dry season, and in certain situations, *malefactor* and *albimana* will be found together in the same streams or pools, but it indicates certain different tendencies in the respective species.

BLOOD FEEDING NECESSARY FOR ANOPHELINES

A blood meal seems to be necessary for the development of the ova of Anophelines. In Experiment No. 40, to determine this point, male and females, *Ce. albimana* and *A. pseudopunctipennis* were placed in a breeding jar and fed on vegetable food and water daily, but they received no blood meals. Upon dissection of females as they died, none showed any development of the ovaries.

PARTHENOGENESIS

If, however, there be given one blood meal the ova may develop even in virgins kept out of contact with males. In the latter instance (with *Stegomyia calopus*) the ova have never developed into larvae.

Experiment.—Virgin Anophelines bred out from single isolated pupae were transferred to one jar entirely out of contact with males. There were three *Ce. albimana* and two *A. pseudopunctipennis*. When applied to the arm all the *Ce. albimana* drew blood; neither of the *A. pseudopunctipennis* would bite. The following day one *A. pseudopunctipennis* died, but the remaining one, with two *Ce. albimana* bit again upon application to the arm. These were added to another jar of virgin females, *Ce. albimana* and *A. pseudopunctipennis*, most of which drew blood readily. Upon dissection none of the *A. pseudopunctipennis* showed any development of the ovaries. One *Ce. albimana*, about fourteen days old, contained ova 0.48 mm long. The single spermathecae of the mosquitos were examined, and in no instance contained spermatozoa. The ova would not develop into larvae in water, and upon microscopic examination were found to contain finely granular food material, but no partly developed larvae, such as would be seen in fertile ova of this size.

A further experiment with *Stegomyia calopus* indicates that whereas ova may develop in size in the ovaries of unfecundated virgins, they are always sterile and never contain partly developed larvae, but granular, undifferentiated protoplasm or food material.

A. November 11 a jar containing five female *Stegomyia calopus* which had been separated individually as pupae, and always out of contact with males, emerged November 9. They were applied to the arm and three bit and drew blood; they were not as voracious as Anophelines, but behaved with the caution and timidity characteristic of *Stegomyia*. After fourteen days these mosquitos had not oviposited. Two were dissected and the spermathecae found to be free from spermatozoa, but their ova were well developed, 0·560 mm. in length, 0·184 mm. in width.

B. Bred out nineteen virgins, *Stegomyia calopus*, in the same manner, from isolated pupae. Upon applying to arm, sixteen out of nineteen bit and apparently drew blood, but there was no sensation of stinging. The next day when applied only three mosquitos bit. These may have been the ones that did not bite the day before. Thirty-seven days after the first biting, eight of these virgins were living, the dead ones having become water-sprawled. Forty black ova were found in the water dish this morning.

41 days after the first biting six ova were found in the dish;

54 days after the first feeding one dead female was found, and upon dissection contained no ova;

69 days after the first feeding a dead female was found to contain twenty well-developed ova, 0·560 mm. in length;

61 days after first feeding forty-three black ova were found in the dish, completely developed, 0·720 mm. in length, 0·240 mm. in width;

but none of these ova developed later into larvae. One hundred and four days after the first feeding the remaining female is still living.

EFFECT OF SALT AND SEA WATER ON ANOPHELINE LARVAE

In general, the effect of an irritating, toxic, or otherwise unusual fluid on mosquito larvae is to hasten pupation. A number of experiments were tried with sea water, salt water and solutions of the heavy metals, and in most instances in the more concentrated solutions, when the larvae were not killed within twenty-four hours, they pupated, and occasionally the period of pupation was shortened; so that if, for instance, in a district sea water were used as a larvacide the first effect would be to hasten pupation and thus increase the number of Anophelines in the district, and if later the sea water became diluted by rain, several species of malaria-transmitting Anophelines might breed in it without difficulty, notably *Ce. albimana* and *Ce. tarsimaculata*. On this account sea water could not be used with any degree of success as a larvacide for Anophelines, except in large quantities and in certain locations.

Chlorine Contents of Natural Waters in which Mosquito Larvae have been taken, and in some instances Bred Out

	Per cent. of Sodium Chloride
A. pseudopunctipennis	0·00165
Ce. albimana	1·93
Anopheles (Sp.)	0·65
Ce. albimana	1·165
Ce. albimana	0·255
Arr. (?) *malefactor*	0·16
Ce. albimana	0·16
Arr. (?) *malefactor*	0·00002
Ce. albimana	0·00125
Ce. tarsimaculata	0·16
—	0·21
—	0·63
—	1·02
Ce. albimana	0·02
A. pseudopunctipennis	0·02
Stegomyia calopus	0·26
Culex (Sp.)	0·057
Aedes taeniorhynchus	2·20

Sea water taken from Panama Bay contained 3 per cent. of sodium chloride, a sample from Simon Bay (Atlantic) contained 3·17 per cent., so that it will be seen from the foregoing table that some Anophelines, under stress of circumstances, may breed in very brackish water.

EXPERIMENTS WITH LARVACIDES

A number of experiments were carried out for the purpose of obtaining a cheap and efficient preparation for destroying mosquito larvae. Crude petroleum oil was frequently too viscid to have a spreading power of the highest efficiency. When mixed with crude carbolic acid, however, its spreading powers were increased.

Much of the crude carbolic acid supplied had been found upon analysis to consist chiefly of inert neutral oils with a small proportion, 5 per cent. to 10 per cent., of tar acids, and as this crude acid was used extensively as a disinfectant, experiments were conducted for the purpose of utilising if possible this crude carbolic acid as a disinfectant and larvacide. It was found that crude carbolic acid, having a specific gravity not greater than 0·96 or 0·97 and containing about 20 per cent. of phenols or tar acids, when made into a soap with common resin and an alkali yielded a product which was an ideal larvacide, having excellent diffusing and toxic powers, and at the same time it was a very efficient germicide. It diffused perfectly with water, forming a milky emulsion very destructive to mosquito larvae, and having a germicidal value of, or greater than, that of pure carbolic acid, or a Rideal-Walker co-efficient of one to two. In this way a very valuable larvacide and disinfectant, miscible with water, was produced from a very inferior disinfectant.

The larvacidal powers when tried with Culicine and Anopheline larvae varied slightly with the quality of the crude carbolic acid, but an average result is as follows:

Dilution 1 to 1000—Culicine larvae, dead in 5 minutes.
Anopheline larvae, half grown, dead in 5 minutes.
Anopheline larvae, full grown, dead in 10 minutes.
Dilution 1 to 5000—Anopheline larvae, half and full grown, dead in 5 minutes.
Culicine larvae, half grown, dead in 3 minutes.
Dilution 1 to 10000—Culicine larvae, half grown, dead in 64 minutes.
Anopheline larvae, young, dead in 52 minutes.
Anopheline larvae, full grown, dead in 135 minutes.
Dilution 1 to 15000—Small Culicine larvae, dead in 32 minutes.
Anopheline larvae, full grown, dead in 123 minutes.

Anopheline larvae seem to be slightly more resistant than Culicine larvae, and all pupae are more resistant to the effects of the larvacide than larvae are.

EXPERIMENTS WITH AGENTS DESTRUCTIVE TO VEGETATION, GRASS AND ALGAE

A series of experiments was carried out with the larvacide, caustic soda, arsenic and copper sulphate as to the amounts necessary in pools and lagoons to prevent the growth of vegetation and to determine the value of the resulting solutions as larvacides. Bermuda grass in sod was made into artificial ponds in large glass moist-jars and flooded with 0·5 per cent. solution of caustic soda, copper sulphate and sulphuric acid. The sod was well soaked with the chemical solution, but the grass remained vigorous in each instance The jars were undisturbed for a period of eighteen days, when a number of Culicine larvae were introduced into the solution of the artificial pools. The larvae were killed within twenty-four hours in the pools containing copper sulphate and sulphuric acid; but those in the pool containing caustic soda remained alive several days. It was concluded from this that none of the above chemicals could be used to advantage in killing gross vegetable matter such as grasses, and none were of special value as larvacides.

An artificial pool as above was flooded with a 0·125 per cent. solution of sodium arsenite. All but three or four of the stalks of grass were killed and overgrown with mould, the wilting effect becoming apparent in forty-eight hours. After nine days, when the grass was quite dead, several Culicine larvae were introduced into the pool and were killed after one hour's exposure. The pool was twice flushed out to rid it of arsenic salt, but the grass showed no further signs of life at the end of thirty-five days. It was concluded from this that a 0·125 per cent. solution is a valuable agent in destroying gross vegetable forms such as grass, and the resulting water in the pool remained effective as a larvacide.

The common, green, filamentous algae, *Spirogyra* and Culicine larvae were introduced into small glass jars, containing various high dilutions of copper sulphate and sodium arsenite. The results of two series of experiments showed that copper sulphate in dilutions up to 1 part in 500,000 is inimical to the growth of this alga. They become greyish-green in colour, shrunken and lose their fresh and crisp appearance. As a larvacide, however, copper sulphate is not destructive in dilutions higher than 1 in 50,000 parts. Sodium arsenite, on the contrary, seems to stimulate the growth of these algae in all dilutions between 1 in 2,500 and 1 in 25,000,000, the algae remaining green and vigorous. As a larvacide, Culicine larvae were destroyed in sodium arsenite dilutions up to 1 in 100,000. The larvacidal powers of sodium arsenite solutions in contact with green algae seem to vary within wide limits, depending probably upon the power of the algae to take the arsenic salt out of solution into its own protoplasm, thus rendering the surrounding solution less larvacidal. It is concluded from this that copper sulphate is more efficient than sodium arsenite as an algacide in high dilutions, but the arsenic salt is a better larvacidal agent. These results are in keeping with our pharmacological knowledge of the effect of copper and arsenic salts in high dilutions on animal and vegetable protoplasm. It would seem, then, that when grass and algae in pools, without outlets, are to be destroyed, sodium arsenite would be of considerable value for this purpose, and would continue to be efficient until washed or drained out and that copper sulphate is a valuable algacidal agent for the destruction of filamentous algae, as *Spirogyra*.

In experiments with the coal tar larvacide in laboratory tanks and under actual conditions the coal tar larvacide was found destructive to grass in dilutions of 10 per cent., the grass turning brown in two or three days and drying in five to six days. When *Spirogyra* was treated with the larvacide, dilutions of 1 to 2,500 were sufficient to kill, while dilutions of 1 to 5,000 and 1 to 10,000 greatly reduced its vigour.

COMPOSITION AND SIZE OF MESH OF WIRE SCREENING

Two extremely important factors in the use of wire screening for protection against mosquitos, are, first, the size of the mesh, and secondly, the chemical composition of the wire used. In regions where it is only necessary or desirable to protect against Anophelines, a No. 16 mesh screening (sixteen holes to the inch) would answer the purpose, and where, as in this region, it is necessary to protect against some of the smaller species, such as *Stegomyia calopus*, a No. 16 mesh would be practically safe, but not absolutely so. The following experiments were conducted to determine the varieties of mosquitos which would, under stress of circumstances, pass through a No. 16 mesh wire screening. Out of several hundred mosquitos eight common species were able to make their escape through a No. 16 mesh wire.

	Sex	No. of specimens escaped
Stegomyia calopus	Males	10
,, ,, ...	Females	6
Culex cubensis	Male	1
,, *rejector*	Male	1
,, *extricator*	Female	1
Aedes angustidittatus	Female	1
Uranotaenia lowii	Female	1

No specimens of *Ce. albimana* or *A. pseudopunctipennis* escaped through No. 16 wire mesh screen, although several hundred were tried. The methods adopted were as follows:—

A. A square wooden box, well ventilated, with fine crinoline gauze screening on two sides and glass on the other two sides, with a central replaceable partition, covered with No. 16 mesh wire screening, was constructed. Several dozen mosquitos at a time, of the above species were liberated on one side of the partition without food or water, and on the opposite side, close to the screen partition, were placed water, banana, candy, sugar and raisins as bait. Only three mosquitos out of several hundreds of several varieties passed through the No. 16 mesh partition under the conditions of the experiment. As the space including the mosquitos was about one-half of a cubic foot in volume, and as there were a few recesses in which the mosquitos could hide, an electric light bulb was hung in such a position at night that the mosquitos would be attracted by it, but this did not favour the passage of mosquitos through the screen. Tobacco fumes were passed into the mosquito compartment with a rubber bulb apparatus, and while this excited the mosquitos, did not cause any of them to escape through the screen. When a person's arm was introduced into the compartment close to the No. 16 mesh wire partition it did not induce the mosquitos to escape through the screening.

B. Next a lantern-chimney, covered on one side with fine mesh crinoline gauze and on the other side with a metal collar holding in place a piece of the No. 16 mesh wire screening, was partly filled with various mosquitos and placed near the same bait as before under a large glass bell jar. Eighteen mosquitos escaped from the chimney through the No. 16 mesh screening into the surrounding jar. The closer quarters and the absence of resting places in the chimney evidently favoured the escape of mosquitos through the wire screening. On one occasion, by passing a gust of air through the lantern chimney jar, a male *Culex* was helped through and escaped.

The conditions in the experiments were all rigid and more extreme than those under actual conditions where mosquitos are trying to enter a screened house from the open.

The chemical composition of various screening material used was investigated by Dr. R. W. Nauss, formerly of this Laboratory. Screening of excellent quality was compared with that which had deteriorated more or less rapidly, and analyses of screens and their incrustations made to determine the factors concerned in its corrosion.

In the investigation considerable attention was paid to the analyses of the efflorescence or incrustation formed on the screening for the determination of the constituents involved in the corrosion. The specimens presenting the highest degrees of deterioration furnished the largest amounts of incrustation. The deterioration of the screening is largely due to the presence of iron in the brass alloy, plus the influence of a hot, moist atmosphere.

Observations continued over a period of four years on screening made of copper and zinc with a composition nearly:—

Copper ...	84·92	89·94	84·83	88·59	95·8
Zinc	—	—	14·90		4·1
Iron	—	—	0·06	0·04	0·0

showed that these resist the corroding actions of a hot, moist climate much better than screening made of brass with an average composition of:—

Copper	65
Zinc	34
Iron	1 +—

and it is concluded that screening intended for use in the tropics, exposed to heat and moisture, should have a high copper content higher than brass, and be as free as possible from the presence of iron.

VALUE OF DAILY COLLECTION AND DESTRUCTION OF LIVE MOSQUITOS CAUGHT IN BARRACKS AND QUARTERS

Abundant material was received for observations on the value of this practice. The daily catch of mosquitos from barracks of various districts would be sent alive to the laboratory. The mosquitos were transferred to breeding jars and fed on dates or raisins until their intestinal tracts were free from blood. They were then killed and examined for zygotes or sporozoites. The species examined were: *Ce. albimana*, *Ce. tarsimaculata*, *Ce. argyrotarsis*, *Arr. (?) malefactor*, and *A. pseudopunctipennis*. A number of specimens of Culicines were also examined at this time. It is noteworthy, and speaks well for the practice of these daily killings, that but one naturally infected Anopheline was found, and that one a specimen of *Ce. argyrotarsis*.

After the first forty-three negative dissections no record was kept of the total number examined, but it was in the neighbourhood of five hundred.

EFFECT OF QUININE ON THE MALARIAL PARASITE IN (*A*) THE MOSQUITO AND (*B*) MAN

A. Nearly all the infecting experiments were conducted on patients who were receiving the routine ward treatment of quinine, grains 10, *ter. die*, in solution, so that apparently quinine in these quantities has no destructive or inhibitive effect on the parasites in the mosquito because the zygotes go on to maturity and sporozoites appear in the salivary glands in from nine to eleven days.

One experiment should be mentioned, however, because the patient received no quinine for several days before the mosquitos became infected and none during the experiment, so that the mosquitos never received any quinine. One *Ce. albimana* contained the rather large number of one hundred and sixty-eight zygotes upon dissection. It should be mentioned as well that from this patient two *A. pseudopunctipennis* became infected. It may be that quinine has a slight inhibitory effect on the parasite in the mosquito's mid-gut.

B. The following tables show the gradual but steady decrease in the number of gametes in the peripheral blood during the administration of quinine, grains 10, *ter. die*, in solution, and one table shows the effect of withholding quinine.

The differential leucocyte counts are tabulated as well, and in these the relative increase in mononuclear elements—lymphocytes, intermediate, and large mononuclear cell—the latter being the chief circulating phagocytic cell in malaria.

As indicative of blood regeneration and the secondary effect on the homopoietic organs, it is interesting to note the increase in the eosinophiles.

CHART 51499

	Dec. 30	Dec. 31	Jan. 2	Jan. 5	Jan. 6	Jan. 8	Jan. 13	Jan. 15	Jan. 23	Feb. 1
Polymorphonuclear	68	65	75	81	65	61·5	65	51	53	66
Large mononuclear	4	16	6	5	6	13·5	13	11	9·5	2·5
Lymphocyte	20	13	13	11	20	16·5	11	14	18	11·5
Large lymphocyte—Intermediate	–	–	–	–	–	—	—	[illegible]	4	7·5
Eosinophile	7	5	5	2	8	7	11	14	14·5	12·5
Mast	1	1	1	1	1	1·5	0	2	1	0
Crescents	67	42	76	46	40	15·5	9	5	0·5	0

The table given above shows the rate of diminution in the number of gametes (crescents) by the administration of quinine, grains 1 *ter. die*, in a Spaniard, 60 years of age, on Isthmus twenty months, whose blood contained numerous crescents but no young forms, and whose temperature was 97° F. continuously. It also shows the degree of change in the proportion of eosinophiles, of increase in mast cells, very slight lymphocytosis and polymorphonuclear decrease.

Compare this with the following: -Case 48,987 of malignant tertian malarial fever, from whom quinine was withheld for twenty-four days. Spaniard, on the Isthmus three months, temperature normal on admission.

1908.	Nov. 11	Nov. 24	Nov. 27	Nov. 30	Dec. 4
Polymorphonuclear ...	86	53	62	42	44
Large mononuclear ...	2	3	22	11	16
Lymphocyte	9	39	14	46	38
Large lymphocyte ...	0	2	2	0	0
Eosinophile	2	3	0	1	2
Mast	1	0	0	0	0
Crescents	16	27	12	8	20

In this case the continuance of the gametes in the peripheral blood is striking. The polymorphonuclear decrease and the lymphocytosis should be noted. This patient received no quinine during the period between November 11 and December 6. Young malignant tertian forms were always present in his blood with gametes. His temperature was irregular, and irregularly quotidian in character.

53,937: Spaniard, on the Isthmus twenty months, temperature normal, blood contained on admission many crescents but no young parasites; quinine, grains 10, *ter. die*, with Fowler's solution, gtts. 5.

CHART No. 53937

	Feb. 5	Feb. 6	Feb. 8	Feb. 9	Feb. 11	Feb. 14	Feb. 15	Feb. 16	Feb. 17	Feb. 1
Polymorphonuclear ...	69	41	41	42	40	52	33	40	39	52·5
Large mononuclear ...	8	33	20	11	19	11	20	18	23	7·5
Lymphocytes	18	17	23	34	28	22·5	24	25	23	23
Large lymphocytes ...	3	8	10	8	5	10·5	6	14	10	7
Mast	0	0	0	0	1	0·5	0·3	0	2	0·5
Eosinophile	2	1	6	5	7	3·5	9	3	3	9·5
Crescents	92	87	61	48	20	3·5	4	3	2	0·5

This table illustrates, as in the preceding one, the steady disappearance of crescents under quinine, grains 10, *ter. die*, also the variation in the proportions of leucocytes.

It should be said that the specimens of blood were always taken at 4.30 or 8.30 p.m., or about four hours after a meal.

53,742: Spaniard, sixteen months on the Isthmus; blood: malignant tertian rings, crescents, ovoides; spleen enlarged to umbilicus. February 1, quinine, grains 10, *ter. die.*; February 6, Fowler's solution, gtt. 5, *ter. die.*

History No. 53742

	Feb. 1	Feb. 2	Feb. 3	Feb. 5	Feb. 6	Feb. 8	Feb. 9	Feb. 11
Polymorphonuclear	56	48	36	44	56	42	43	42
Large mononuclear	17	22	16	15	12	18	25	27
Lymphocytes	15	21	27	24	8	18	14	12
Large lymphocytes	8	4	3	8	10	12	8	9
Eosinophile	3	5	15	7	13	9	10	9
Mast	1	0	2	1	1	1	0	1
Pigmented leucocytes	—	—	1	1	—	—	—	—
Crescents	29	22	29	14	10	5	5	2

Notes:—Blood, February 2, 6,500 leucocytes per mm.^{3}.
Blood, February 5, containing one phagocyted gamete.

The large mononuclear increase is striking.

M. L., Spaniard, malignant tertian malaria, returned and died. Autopsy, March 28, 1909. Quinine grains 10, *ter. die.*

	Dec. 26	Dec. 27	Dec. 28	Dec. 30	Dec. 31	Jan. 2	Jan. 13
Polymorphonuclear	48	65	60	64	70	65	45
Large mononuclear	13	6	5	9	3	12	5
Lymphocyte	34	25	32	14	22	14	34
Eosinophile	4	4	3	12	5	8	16
Mast	—	—	—	1	—	1	—
Pigmented leucocyte	1	—	—	—	—	—	—
Crescents	9	5	5	4	1	0	0

Note:—Poikilocytosis and basophilia of red blood corpuscles on January 13.

The following two records are taken from cases of simple tertian malaria receiving quinine, grains 10, *ter. die.*

HISTORY No. 51147

	Dec. 21	Dec. 23	Dec. 26
Polymorphonuclear	69	18	48
Large mononuclear	7	39	12
Lymphocyte	23	36	[illegible]
Mast	1	—	—
Eosinophile	0	7	[illegible]
Gametes	3	1	

Quinine discontinued after an initial dose.

HISTORY No. 50792

	Dec. 14	Dec. 15	Dec. 17	Dec. 20
Polymorphonuclear	64	48	17	23·5
Mononuclear	19	17	28	24
Lymphocyte	7	15	45	41
Eosinophile	10	19	8	10
Mast	0	1	1	0·5
Pigmented leucocyte	0	0	1	0·5
Gametes	?	?	0	0

Quinine discontinued after an initial dose. Returned January 11 with malignant tertian malaria—seven crescents per hundred leucocytes.

HOSPITAL No. 50782

	Dec. 14	Dec. 15	Dec. 17	Dec. 20	Jan. 13
Polymorphonuclear	47	40	20	47	42
Large mononuclear	34	34	39	13	11
Lymphocyte	16	26	37	37	42
Eosinophile	3	0	4	2	4
Mast	0	0	0	1	—
*Gametes	0	0	0	0	—

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*None of seven *Cc. albimana* became infected from this case, indicating the absence of gametes.

In each of the above cases the rapid polymorphonuclear decrease and the equally rapid mononuclear increase should be noted.

The effect of quinine administration, then, is to make the gametes gradually disappear from the peripheral blood by the destruction of the young forms, the gametes being phagocyted by splenic and hepatic endothelium. It is concluded that quinine, grains 10, *ter. die*, in solution, will gradually reduce the sexual form of the parasite in man to a non-infective minimum in from a few days to a few weeks, depending on the severity of the infection.

In simple tertian malarial fever, gametes disappear from the peripheral blood within two or three days under quinine treatment, and generally disappear even when quinine is withheld, if the patient is at rest. There are never as many gametes in the peripheral blood in simple tertian as in malignant tertian malaria. As a consequence, one never finds as many simple tertian zygotes as malignant tertian zygotes in infected mosquitos.

PRELIMINARY EXPERIMENTS ON THE EFFECT OF COLD ON VARIOUS DISEASES IN SMALL ANIMALS

BY
PROFESSOR MAJOR RONALD ROSS, C.B., F.R.S.,
AND
MAJOR C. L. WILLIAMS, I.M.S. (RETIRED)

(Received for publication 11 June, 1910)

Prefatory Note by R. Ross

Some years ago Sir Edwin Durning-Lawrence, Bart., offered funds to the Liverpool School of Tropical Medicine for the purpose of experimenting on the effect of cold on Yellow Fever—a subject in which he had long been interested owing to various reports which had come to his knowledge. The School found it difficult to give effect to his wishes, and the matter was allowed to drop.

For a long time I have felt that much experimental work still remains to be done regarding the cure of parasitic diseases, especially by such simple natural agencies as heat and cold. Most of the animal parasites occur most frequently in persons who live in warm climates, that is to say, they are accustomed to live in hosts who themselves live under conditions favourable to the said parasites. There may be reasons for thinking that if these conditions are abruptly altered the result will be harmful to the parasites, just as abrupt changes are apt to be harmful to higher organisms. Of course, the temperature of the patient's body is not markedly changed by alteration of external temperature; but, nevertheless, certain changes may be produced in his blood or tissues which are likely to be inimical to parasites living in him. I cannot point to any very definite prima facie evidence in favour of this view, but some general observations may be mentioned. For example, it is a common custom to send patients suffering from malaria to temperate climates, as to the hills in India. From my own clinical experience I certainly think it is easier to treat malaria in

England than in the tropics, and easier to treat it in the cool season in the tropics than in the very hot season —that is to say, I think that a given dose of quinine will produce least effect in the hot climates in which the parasites are most easily propagated. Moreover, if we make a careful study of statistics in India, we often observe a sudden rise in the malaria rate at the end of the cold season, long before any new brood of Anophelines has hatched out. True, this rise may be partly explained by the emergence of old Anophelines from hibernation; but it may also be due to relapses caused by the greater heat. There are also many observations on record, especially in Italy, suggesting that season affects the plasmodia, especially the sexual forms, quite apart from the proliferation of the carrying agents. Human trypanosomiasis occurs in persons in very hot climates, and may just possibly be retarded if such persons are removed to cold ones. In one case of complete cure known to me, marked improvement occurred when the patient was subjected to considerable cold, accompanied by healthy exercise and open air in Scotland. Dysentery seems to improve much more rapidly in cool climates than in hot ones. Possibly the effect may only be a slight one; but it is well worth while to study whether there is any effect at all, and if so how much—because anything that concerns so important a matter as the treatment of disease is worth considering. Still greater improvement might be effected in many maladies, especially diseases of the respiratory system, and of the skin, if cold is combined with a very dry and pure atmosphere, and, of course, with other forms of treatment.

Last year, therefore, I asked Sir Edwin Durning-Lawrence whether he would not assist us to carry out researches on these points in Liverpool. He assented at once, and suggested that I should ask his friend, Sir Alfred Haslam, the head of the great refrigerating works at Derby to construct a chamber capable of being kept at the requisite temperature. The result was that the Haslam Engineering Company made such a chamber at the University Laboratories of this School It was completed on September 10th, 1909, and after a few interruptions, has been kept continuously running ever since. Major C. L Williams was put in charge of the experiments from early in September until the 13th April, 1910, since when the work has been conducted by Dr. J. G. Thomson. At the time when Dr. J. G.

Thomson commenced his labours, Dr. David Thomson and myself had just elaborated what we call Enumerative Methods* for the study of parasitic diseases, and these have now been employed for the Cryotherapy work. In this paper, however, we record only the preliminary experiments conducted by the old methods—which were the only ones used by Major Williams.

Our warm thanks are due to Sir Edwin Durning-Lawrence for his munificence regarding this extensive line of research, and to Sir Alfred Haslam for the great interest which he has taken in the matter.

Summary of Experiments from the 10th September, 1909, to 13th April, 1910

By Major C. L. Williams, I.M.S.

The chamber is 12 feet long by 7 feet wide by 6¾ feet high, with a cubic content of about 540 feet, and can be kept at any temperature between about 15° F. (−9·4° C.) and 150° F. (65·5° C.). The motor was worked usually from about 7.45 a.m. to 5.30 p.m., but had to be stopped at intervals, perhaps for an hour or two at a time whenever 20° F. was reached. Usually it rose during the night to 36° or 38° F., or thereabouts, and that would be the initial temperature at 7.30 a.m. at the time of starting the machinery. The temperature was reduced by an ammonia compressor worked by a 6-h.p. motor and a fan driving in air through a chamber in which a saturated solution of calcium chloride was kept constantly trickling over corrugated iron plates.

In theory the air is not supposed to change or be refreshed by additions of outside air, but apart from the rush of air into the chamber each time the door was opened, a procedure necessary several times a day, the absence of smell and the practically inappreciable amount of CO_2 and organic matter in the air when tested chemically point very strongly to a rapid removal of respiration products: and, moreover, the animals in the chamber at no time showed any signs of intoxication by impurities of re-breathed air.

The low humidity of the air in the chamber is no doubt a strong factor in the results. It varied usually from about 50 to 60 per cent.,

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* Vide page 261 of this number.

TABLE SHEWING THE COURSE OF VARIOUS TRYPANOSOMIASES UNDER ORDINARY AND UNDER COLD CHAMBER CONDITIONS UP TO FEBRUARY 21, 1910

Trypanosome	Animal infected	Number of animals			Incubation period in days			Period lived in days			Failed to shew infection	Remarks
					Longest	Shortest	Average	Longest	Shortest	Average		
T. lewisi ...	Rat ...	13	Cold chamber	6	25	4	14	127 (Still alive)	20	40	2	One still alive (21.2.10) shewed trypanosomes for 35 days. Its weight has risen from 85 to 163 grams in 127 days its coat, however, is much longer, and it was probably young when received). Two died after 45 and 53 days, of epidemic pneumonia.
			Controls	7	12	4	8	112 (Still alive)	21	46	3	Two died after 12 and 69 days respectively of epidemic pneumonia. One is still alive.
T. equiperdum...	Rat ...	28	Cold chamber	14	20	6	13·4	36	12	25	1	Note that the sole failure was in the cold chamber ? aborted infection). One died soon after inoculation.
			Controls	14	24	8	17·8	38	21	26·8	—	
T. brucei ...	Guinea-pig	15	Cold chamber	8	36	4	18	62	20	37·1	—	One died soon after inoculation.
			Controls	7	35	5	17·1	57	20	34·1	—	
T. evansi ...	Guinea-pig	6	Cold chamber	3	21	18	19·5	26	24	25	1	
			Controls	3	10	Not known	9	21	Not known	21	1	One died in five days (? cause), before any trypanosomes had shewn in the peripheral blood.

NOTE.—Some of these animals were removed for ten days to the animal house whilst the cold chamber was under repair, and for that amount of time were subject only to ordinary temperatures.

NOTE.—One 'lewisi' rat is still alive [illegible] in the cold chamber, and [illegible] lively and well. It has [illegible] trypanosomes.

being higher on days when the humidity outside was high, and being raised each time the door had to be opened to admit of feeding, taking observations, etc. To the observer it proved very invigorating, comparable probably to the air in the interior of Canada. The animals in the chamber, too, seemed more active and more interested in their surroundings than the controls which were kept in a greenhouse, artificially heated, but with a very varying temperature and with the humidity of the outside air. Curiously enough, the animals in the control house threw out actually longer coats than those in the cold chamber, and their appetites were markedly less.

The animals subjected to the influence of the cold were guinea-pigs, rats and mice. The animals were placed in cages, well bedded and well fed, fat and proteid especially being provided in their diet.

The diseases brought under the influence of the cold were chiefly various trypanosomiases, with, in addition, tubercle (bovine), cancer (in mice), tetanus, and spirochaetosis, and the results during over eight months' observation are tabulated below. During the eight months there were occasional intermissions for a few days for repairs to machinery, etc., but they amounted to but quite a few days in all.

In the table it will be noted that four animals died of pneumonia, an epidemic of which, unfortunately, occurred at one period and carried off several of the animals under observation. It was probably introduced with some wild rats placed at first near the controls, but affected both controls and cold chamber subjects equally.

The influence of *T. lewisi* is, in any case, difficult to ascertain as rats recover spontaneously from this affection; but it will be noted that the only animal which failed to show trypanosomes after inoculation with *T. brucei* was one in the cold chamber. The dose in all cases was, of course, the same, in terms of c.c. for the animal observed and its control, but the actual number of trypanosomes injected must vary within very wide limits, and this factor will influence the incubation periods. Whereas it seems to be somewhat delayed in *T. lewisi* it varies but little in the other trypanosomes. There was a small prolongation of life in the Ngana and Caderas cases, but this did not hold good for *T. lewisi* and Dourine.

Twelve mice were inoculated with cancer, and six put into the cold chamber; five lived 50, 51, 56, 70 and 90 days respectively, whereas of their six controls, two lived 56 and 76 days respectively.

Of the remainder, three controls failed to develop tumours, and one each in the control and cold chambers died within a few days of inoculation.

Six mice were also put into the cold chamber uninoculated as controls, and of them two are still alive (21 Feb.), well and vigorous after 120 days, showing that cold *per se* had no bad effect on them, whilst two were eaten by their comrades after respectively 24 and 42 days' residence in the cold chamber, and two died of natural causes in 56 and 62 days.

Some mice were also infected with *Spirochaeta duttoni*, but no appreciable influence on the course of this disease could be observed in them.

Five guinea-pigs with tetanus were placed under observation, a case of a boy cured of that disease whilst being treated in a refrigerator having been reported by Crane ('St. Louis Medical Review,' 7th July, 1906). Of the tetanus guinea-pigs, one injected with $\frac{1}{500}$ c.c. of a culture and placed in the cold chamber, died in 9½ days, but it is very doubtful if it died of tetanus: two injected with $\frac{1}{1500}$ and $\frac{1}{1000}$ c.c. respectively and kept as controls never showed any symptoms; whilst two injected with $\frac{1}{500}$ c.c. and placed one in the cold chamber and one as control died with similar symptoms in a similar time—something over 50 hours.

An Englishman suffering from sleeping sickness of some three months' duration, and contracted in N. Rhodesia, was submitted to a course of treatment in the chamber from January 5 onwards to the end of that month. Ordinarily he came from the hospital in which he was by cab about 10 a.m., and rested in the chamber, well wrapped up, till about 3.30 p.m. In all he had a course (interrupted for some days) of twelve days, or some 53 hours in all, of cold air. The temperature was gradually decreased till 24° F. was reached. He himself said he felt much better for the treatment, which acted on him as a tonic; but in the short period he was under observation no visible diminution was apparent in the number of trypanosomes in the blood. He was getting atoxyl simultaneously at his hospital. Several times he walked back—a distance of fully 1½ miles—after his cold chamber rest; but since February 1 he has not been able to leave the Hospital, and has therefore been unable to utilise the action of cold in altering his metabolism, and thereby the culture media within his body of *T. gambiense*.

As regards increase or loss of weight in the animals under observation, most of them were young, and their weights, of course, increased by mere growth. Heavier coats also added to the weight. Of those on which accurate data can be founded the results are as follow:—Number of animals: Rats, 19; guinea-pigs, 8. Rats: Gained weight, 14; lost weight, 5. Guinea-pigs: Gained weight, 6; lost weight, 2.

Animal	Cold Chamber		Controls		Total
	Gained weight	Lost weight	Gained weight	Lost weight	
T. lewisi (rats)	3	1	1	1	6
T. equiperdum (rats)	6	1	4	2	13
T. brucei (guinea-pigs)	3	—	2	—	5
Tubercle	—	1	1	—	2
T. evansi	-		-	1	1
Total	12	3	8	4	27

Put shortly, all the animals in the cold chamber gained weight except three, of which one was a case of tubercle: showing, on the whole, and as far as the small figures go, a favourable influence on their general nutrition. But these figures are amply supported by the obvious increase in weight of practically all the animals in the chamber, though their immaturity in most cases makes the actual results in grams of weight gained of less value from the point of view of influence of cold *per se*.

Finally, as regards the temperatures of the animals, no constant effect could be observed in the influence of the cold chamber, and those in and those out varied equally irregularly, with no permanent ratio between the one and the other.

Note on 12 April, 1910. The result of further experiments carried out since February 21 and bringing the observation period up to over eight months is as follows:—

Six guinea-pigs were inoculated with bovine tubercle (0·1 mgr. culture) on the 27th November, and three placed in the cold chamber and three outside, with, of course, uninoculated controls in both cases. The uninoculated controls throve and call for no remark, except that one in the cold chamber died after 34 days of no obvious cause, there being only a little lung congestion evident in post mortem. Of the inoculated, all are still alive, and those in the cold chamber have now been in it 137 days, and seem quite fit and well. One female has aborted twice, but whether from the cold, the tubercle, or other cause, cannot be definitely stated. Only three were apparently mature at the time of inoculation, and their weights in the case of two inoculated have risen in one case from 400 to 653 grams, and fallen in the other from 772 to 747; whilst the third, uninoculated, has risen from 622 to 794. Their temperatures have not shown any very striking variations; but the swelling over the site of inoculation burst fully a month sooner in the two controls outside, in which rupture has occurred, than in the one in the cold chamber, which developed suppuration; and this, as far as it goes, points to some diminution of the virulence of the bacilli in the cold chamber.

Four guinea-pigs have since (22 March) been inoculated with human tubercle, and three of them are at present under observation, one having died soon after inoculation.

Six guinea-pigs were inoculated with *T. gambiense* on the 14th December, and, as usual, placed three in the cold chamber and three outside as controls. All failed to show trypanosomes in the peripheral blood. One in the cold chamber died 16 days after inoculation, but no trypanosomes were found in the heart's blood post mortem, and it probably died of other causes. The other five were again inoculated on the 4th March, and again failed to show trypanosomes. But a third inoculation on March 30 has been successful and interesting, inasmuch as, whereas the three outside showed parasites in their peripheral bloods on the fifth day, of the two inside one only showed them very scantily on the eighth day, and has not shown them since, the other has so far not shown them at all: a result pointing strongly towards at least a delayed development in those in the colder air.

Several rats have been inoculated with cultures of pneumococcus, but so far no marked difference has been observable in their symptoms, whether in or out of the chamber, and none have died so that they remain under observation, and the final result will only become apparent later.

MALARIA PREVENTION IN JAMAICA

BY

SIR RUBERT BOYCE, F.R.S.,
DEAN OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(Received for publication 16 June, 1910)

PLATES XVI-XVII

Although it is too early to sum up the results of the special Commission appointed in October to enquire into the malarial problem in Jamaica, nevertheless, as the methods of procedure of the Commission are of real and practical interest at a time when it is incumbent upon all colonies to take steps to abate the malaria nuisance, I have considered the narration of the following facts not without interest, as I have had myself an opportunity of examining the work in progress, and as an important paper dealing with the subject of malaria in Jamaica has appeared in a previous number of these 'Annals.'*

Appointment of the Commission

The Commission was appointed by Sir Sydney Olivier, K.C.M.G., the Governor, on October 16, 1909, and consisted of the following representative members:—

The Colonial Secretary, P. C. Cork,
The Archbishop of the West Indies,
The Senior Medical Officer, Dr. Kerr,
Dr. Turton,
Dr. Grabham, Entomologist,
Messrs. Gideon and Hurt.

Mr. F. N. Isaacs was subsequently appointed Secretary. The selection of the Commission was a wise one, the members being fully alive to the importance of the work—sanitarians of pronounced modern views, and in several instances gentlemen who had contributed valuable papers upon the subject, or who had had previous experience of anti-malarial work in other colonies.

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* Annals of Trop. Med. and Parasit. Vol. III, No. 4.

Power and scope of the Commission

The Commission had considerable power and a comparatively free hand. They were directed to conduct investigations, to summon witnesses if necessary, employ inspectors and experts to assist and advise them. They were, furthermore, empowered to carry out remedial and preventive works for the abatement of malaria independent of local sanitary bodies, and to conduct medical and biological investigations and to report from time to time. They were authorised to spend for scientific and preventive measures a sum in the first instance not exceeding £500 without obtaining further authority. They were also asked to report upon, and to devise measures to prevent other diseases which they might come across in the course of their investigations.

First steps of the Commission

This consisted in ascertaining the prevalence and distribution of malaria in the Island. In the first place the Commission paid a just tribute to the Report upon Malaria in Jamaica drawn up by Dr. Prout,* in which both the causes of malaria and the principles necessary for its control were fully discussed.

In the second place they asked for returns of all the cases of malaria in the island, and Dr. Grabham, one of the members of the Commission, took a splenic census of the West end district of Kingston. The Secretary, Mr. Isaacs, proceeded to Annotto Bay to make a survey of the town and district in order to ascertain the anti-malarial measures which could with advantage be introduced. Similarly the Black River, Yallahs, Morant Bay and Bath districts were visited and inspected for breeding places of Anophelines, in order to devise methods for their reduction.

From these preliminary investigations the Commissioners were able to report in February, 1910, that one-half the Island was practically free from malaria-carrying mosquitos. This area comprised those parts of the Island above 1,000 feet. Below 1,000 feet the Anophelines were found in varying numbers, and were greatest in the low-lying plains near the sea coast, where natural drainage was most difficult. Breeding places existed in the East and West end districts of Kingston and the West End constituted a badly infected endemic centre; a splenic census taken by Dr. Grabham in December, 1909, disclosing a malaria rate of 65 per cent. The cases which were received into the Kingston Hospital came mainly from this district. In this district also investigations disclosed the interesting fact that the breeding places were to a very large extent artificial, and therefore preventable collections of water, mainly due to the abuse of the filtered water supply of the town and to neglected drains and gullies. In company with Mr. Isaacs, I visited this district in April of this year—1910—and observed for myself a miniature but complete system of irrigation consisting of streams, rills, ponds and pools, all fed and kept up by the simple device of turning the taps of the domestic water supply full on, night and day. This method yielded an abundant supply of water, and enabled an extensive and profitable market-gardening system to be carried on in the midst of Kingston. Looking down upon the town, the district in question could be readily picked out by reason of its conspicuous green appearance, the result of the growth of banana trees and vegetation generally. The houses of the coolies and others who were the principal cultivators in the district were very numerous, and in these the Anophelines kept up an abundant supply of malaria cases, and helped to spread the disease to wider areas around. Thus Kingston presented a picture, of on the whole, a well-planned and drained town, from which yellow fever and malaria had been driven out and were no longer endemic; but in it there existed a comparatively small, thickly-populated area where malaria was still endemic, and the cause of the formidable number of cases of malaria entered on the books of the Public Hospital. A more striking and instructive picture of the inter-relationship of Anophelines and their breeding places with the prevalence of malaria could probably not be easily paralleled in a similar area anywhere. It serves to conclusively demonstrate the utility and importance of making investigations. The unnecessary suffering and expenditure of money caused by this endemic focus were naturally considerable, and the wanton waste of money caused by utilising the *filtered* town water supply for market-gardening purposes has a comical aspect were it not associated with such serious consequences. The Commissioners were not slow in pointing out how readily this glaring abuse might be remedied, and how by the construction of better drains and by more vigorous filling-in operations this endemic focus might be rendered malaria-free. The photographs which I have had taken and which are inserted in this paper, show very conclusively the extent of Anopheline breeding grounds which the abuse of the domestic water supply can bring about, if not properly controlled.

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* *Loc. cit.*

The Commission started filling-in operations in the offending district, and took steps to distribute quinine at cost price to the authorities of all malarial districts. They also arranged for the training of sanitary inspectors, so that the latter could be in a position to ferret out breeding places; Dr. Grabham took on himself this part of the work of the Commission. Public lectures were also delivered at various centres throughout the Island. In conclusion, the Commission has in a short space of time done good work: it has ventilated the subject of malaria; it has placed its finger upon the offending spots; it has shown with what comparative ease and at what small cost the remedy can be obtained; and it has commenced to enforce the remedy.

Tables showing the deaths from malaria in the various parishes from the year 1897 are appended.

DEATHS FROM MALARIA

Parish	1897-98	1898-99	1899-1900	1900-01	1901-02	1902-03	1903-04	1904-05	1905-06	1906-07	1907-08	1908-09
Kingston	68	46	50	31	71	50	42	47	88	89	89	54†
St. Andrew	18	15	20	15	25	27	16	18	26	18	37	10
St. Thomas	3	7	6	6	9	33	41	17	35	46	72	57†
Portland	34	46	85	40	39	71	124	100	77	87	112	105†
St. Mary	2	4	15	7	5	34	72	40	35	36	49	22
St. Ann	6	3	7	4	1	11	10	8	7	10	20	18
Trelawny	18	5	2	9	1	5	3	10	10	4	12	7
St. James	10	9	5	7	12	12	12	14	18	12	14	12
Hanover	2	1	—	2	1	4	1	—	3	2	1	4
Westmoreland ..	10	3	1	3	4	28	20	22	20	38	28	13
St. Elizabeth	3	4	3	5	7	10	10	9	15	23	23	25†
Manchester	1	1	—	3	—	4	7	2	4	10	14	7
Clarendon	14	7	11	7	14	38	31	36	33	43	34	23
St. Catherine ...	8	10	6	2	12	41	80	84	90	94	165	109†
Whole Island ...	197	161	211	141	201	368	469	407	461	512	670	466

Death Rates for 'Malaria' for the years 1897-98 to 1908-1909

Year	Malaria			
	Rate per 100 deaths		Rate per 1000 of population	
	Parish of Kingston	Whole Island excluding Kingston	Parish of Kingston	Whole Island excluding Kingston
1897-1898	4·5	0·8	1·3	0·1
1898-1899	3·2	0·8	0·8	0·1
1899-1900	3·4	1·0	0·9	0·7
1900-1901	2·1	0·7	0·5	0·1
1901-1902	4·8	0·8	1·3	0·1
1902-1903	3·6	2·2	0·9	0·4
1903-1904	2·6	2·3	0·7	0·5
1904-1905	2·9	1·9	0·8	0·4
1905-1906	5·6	2·2	1·6	0·4
1906-1907	4·3	2·1	1·6	0·5
1907-1908	4·5	2·6	1·6	0·7
1908-1909	2·9	2·4	0·9	0·5

PLATES XVI-XVII

Anopheline Breeding-pools in Jamaica

P P Press, imp.

PLATE XVII.

P P Press, imp.

ON SOME SPECIES OF *CYCLOPS* AND OTHER ENTOMOSTRACA COLLECTED BY DR. J. M. DALZIEL IN NORTHERN NIGERIA

BY

G. STEWARDSON BRADY, M.D., LL.D., D.Sc., F.R.S.

(Received for publication 21 June, 1910)

PLATES XVIII-XX

The species here described were collected by Dr. Dalziel at Yola in Northern Nigeria, in the course of a research on the Life History of the Guinea Worm. The specimens were sent to me for description by Mr. J. H. Ashworth, of the Zoological Department of Edinburgh University, and examples, as far as possible, of the various species have been sent to the University for future reference. Some few types are, however, unavoidably absent. Dr. Dalziel made careful notes of the various localities from which his specimens were taken, which I here transcribe. The sources of each particular species are indicated by the letters affixed to the descriptions.

Sources.

A. Turbid bush-pools with muddy bottom, frequented by cattle and containing fish.

B. Surface-well in clay, free from water plants, but with grass and weeds dipping down the sides to the water's edge.

C. Marshes or pools of clear water when undisturbed; bottom of mud or of grass coated with sediment; containing *Lemna*, *Nymphoea* and other water plants, and fringed with rank grass.

D. Small pools of clear water in shrinking bed of River Benué, recently isolated and therefore not long stagnant, and containing flocculent viscid algae, insect larvae, etc.

E. Benué River, small bays and backwaters of clear water but no current, sandy edges and bottom with some vegetable débris and sediment, but no growing vegetation.

It may be useful to future workers in this field to note here some of the points which need special attention in determining the various species of *Cyclops*.

1. The number of joints in the anterior antennae and the length of the limb relatively to the body of the animal.
2. The numbers of joints in the rami of the four pairs of natatory feet.
3. The characters of the rudimentary fifth pair of feet.
4. The characters and proportional length of the caudal rami.

The general outline of the body and its various segments should be noted, and in living specimens the colours of the body and egg-sacs may provide useful characters.

Dr. Dalziel's attention seems to have been almost entirely directed to the Cyclopidae as being probably the intermediate bearers of human parasites, but it is quite likely that these hosts might also be found among the Ostracoda. In two, at least, of the British species of that group I have myself found scolices of an undetermined species of Taenia, and in yet another Ostracod many specimens of a larval Trematode worm, as well as a fully developed worm belonging to the group Acanthocephala. A brief reference to these may be found in my paper on the British species of Candoninae. (Proceedings of the Zoological Society of London, 1910, Part I.)

COPEPODA

Clycops nigeriae, n. sp. Plate XVIII, figs. 1-7.

Female, length 0·88 mm. Body robust (fig. 1), cephalic segment as broad as long, rounded and slightly produced in front; the two following segments expanded laterally and obtusely angulated behind; last thoracic segment very small; urosome rather short and stout, about one-third as long as the anterior portion of the body, genital segment moderately dilated; caudal rami as long as the united lengths of the last two segments, slightly tapering distally, seta of the outer margin attached rather behind the middle, apical setae long, the innermost considerably longer than the entire urosome (fig. 3). Anterior antennae (fig. 4) eleven-jointed, reaching when reflexed to the middle of the second body-segment, rather sparingly clothed with setae of moderate length. Natatory feet short and stout, with both rami bi-articulate (fig. 6); last pair of feet two-jointed (fig. 2), the basal joint short and not very distinct, apical joint slender, bearing two long setae, the distal one being needle-shaped. Colour stated by Dr. Dalziel to be 'greyish green, eye, deep red.' The general characters of this species are very similar to those of *C. gracilis*, Lilljiborg, and *C. bicolor*, G. O. Sars, and the natatory feet are not unlike those of *C. pachycomus*, one of the many species described by the latter author from Lake Tanganyika.

Dr. Dalziel's specimens are from several different sources. The species would seem to be generally distributed in the region investigated by him. Sources A. B. C. D. E.

Cyclops virescens, G. S. Brady. Plate XVIII, figs. 8-16.

Female, length 0·65 mm. Body slender, the anterior segment ovoid in form, and scarcely at all produced in front (fig. 8), the other thoracic segments not expanded laterally, last segment short (fig. 10); urosome slender, genital segment equal in length to the two following segments, scarcely at all dilated (fig. 10), caudal rami short, about equal in length to the last tail segment, not at all divergent. Anterior antennae (fig. 9), eleven-jointed, reaching when reflexed to the posterior border of the third body segment, slender and sparingly setiferous; natatory feet (figs. 11, 15, 16), with both branches three-jointed; fifth pair of feet uni-articulate and bearing two apical setae (fig. 14). First segment of the urosome in the *male* produced ventrally into a slender spine (fig. 13).

This, like the preceding species, occurred in various gatherings, and Dr. Dalziel's notes assign to it a wide range of colour, from yellow to green and brown. Sources A. B. C. D. E.

There can, I think, be little doubt as to identifying Dr. Dalziel's specimens with those already imperfectly described by me from Dr. Graham's collection under the specific name of *virescens*. Dr. Graham's notes, made from an examination of freshly gathered specimens, assign ten joints to the anterior antennae, whereas the number of joints in Dr. Dalziel's specimens is, so far as I can make out, eleven. But the jointing, as seen in spirit-specimens, is very indistinct, and in any case the slight difference might possibly depend upon the age of the animals. In all other respects the two forms agree with each other.

I am indebted to Dr. Calman, of the British Museum, for the opportunity of examining the type specimens of *C. virescens*, and it is on this that the foregoing remarks are based.

Cyclops longistylis ?, G. S. Brady. Plate XIX, figs. 17-23.

Cyclops longistylis, Brady. Notes on Dr. Graham's collection of Cyclopidae. (Annals of Tropical Medicine and Parasitology, Vol. 1, No. 3, 1907.)

Female, length 0·77 mm. Body slender, tapering gradually from before backwards (fig. 17), first segment longer than broad, sub-ovoid, the three following segments scarcely at all produced laterally, last thoracic segment very small; urosome slender, genital segment not at all tumid, caudal rami very slender, about six times as long as broad, scarcely divergent; anterior antennae ten-jointed, when reflexed somewhat shorter than the first body segment (fig. 22). All the natatory feet have both branches three-jointed, feet of the fifth pair very minute, almost obsolete, bi-articulate (fig. 23), the first joint bearing two setae, the distal joint one seta; attached to this pedigerous segment just in front of the foot are three rather strong setae—longer than the foot itself. The spines of the second and third pairs of feet are in the *male* unusually strong (fig. 19), those of the fourth pair not quite so strong, and those of the first pair almost normal (fig. 20).

The specimens here described were found in Dr. Dalziel's gatherings from the Benué River (Source E), 'colour dark green or greenish-yellow, the egg masses brown, carried close to the body.

The reference of these to *C. longistylis* is somewhat doubtful, and the type-specimens being now in the British Museum—very few in number, and in an imperfect condition—I am unable to verify the reference. The principal doubt arises from a discrepancy in the recognisable number of joints in the anterior antennae, Dr. Graham's record of twelve joints disagreeing with my own observation of ten joints only in Dr. Dalziel's specimens. But these details are not easily observed in spirit-specimens, and need not be absolutely insisted on.

Cyclops leuckarti, Claus. Sources A. B. C. D. E.

Cyclops bicolor, G. O. Sars. Sources A. C.

Cyclops brevipes, n. sp. Plate XX, figs. 31-34.

Female, length 0·55 mm. Body slender (fig. 31). the first segment occupying not much less than half of its entire length, rounded and somewhat narrowed in front, the hinder segments not expanded laterally; urosome slender (fig. 33), the genital segment not dilated and scarcely longer than the next following segment, the first two segments armed with spine-like setae on their distal outer angles, last segment having its distal margin finely aculeate; caudal rami rather longer than the last abdominal segment; setae of the outer margin attached near the middle; apical setae of moderate length. Anterior antennae (fig. 34) eight-jointed, barely half as long as the first segment of the body, sparingly beset with short setae. Natatory feet short, having both rami two-jointed (fig. 34); the place of the fifth pair occupied by two short setae.

This species occurred in the same localities as those recorded for *C. nigeriae*. It is the smallest which has ever come under my notice. The characters of the urosome and anterior antennae sufficiently distinguish it from any other form.

Diaptomus nigerianus, n. sp. Plate XIX, figs. 24-30.

Female, length 1·1 mm. Anterior division of the body rather slender, of nearly equal width throughout, the posterior segment somewhat produced laterally and sharply angulated (fig. 28); urosome (fig. 25) slender, the genital segment not at all protuberant in front, the last two segments together scarcely half as long as the genital segment, and very imperfectly separated from each other; caudal rami dilated distally, scarcely as long as the two anterior coalescent segments, terminal setae broad, sub-spathulate and rather densely plumose, about twice as long as the rami themselves (fig. 25). Anterior antennae slender, scarcely exceeding the entire length of the body; posterior maxillipeds of the usual form, the distal joint exceedingly small (fig. 26); inner ramus of the last pair of legs (fig. 27) simple, much shorter than the first joint of the outer ramus; claw of the outer ramus stout, non-ciliated, terminal joint indistinct.

Male, last segment of the cephalothorax rounded off posteriorly; abdomen narrow, five-jointed; penultimate joint of the right anterior antenna bearing an apical spine, which is shorter than the next following joint (fig. 24); outer branch of the last pair of legs (fig. 30) of the right side simple, the last joint bearing an apical simply-curved claw, and on the outer margin a small papilla and a slender curved spine; the basal joint of the foot of the left side is produced internally into a short digitiform process or rudimentary inner branch; the outer ramus short, bi-articulate, the last joint bearing a small setiferous papilla. The protopodite bears on its distal margin a couple of papilliform processes which overlap slightly the basal joint of the limb. The tail setae of the male are much more slender than those of the opposite sex, and are non-plumose.

This species seems to be more nearly allied to *D. galeboides*, G. O. Sars, than to any other described species, though whether that form be really specifically distinct from *D. galebi* of Mràzek may perhaps be doubted. The types of *D. galebi* were taken in Egypt, those of *D. galeboides* in Lake Tanganyika.

Dr. Dalziel's specimens, here described, are from 'a rocky pool of a hill-stream, *7*. II. 09.'

OSTRACODA

Cypris subovata, n. sp. Plate XX, figs. 35-39.

Shell subovate, tumid; seen from the side subreniform, highest in the middle, height more than equal to half the length, extremities well rounded, the anterior being the narrower of the two, dorsal margin gently arched, ventral slightly sinuated in the middle (fig. 35); seen dorsally the outline is ovate, tumid, greatest width behind the middle and equal to considerably more than half the length (fig. 36), extremities obtusely pointed, the left valve larger than the right and distinctly overlapping in front; shell surface smooth, clothed at the extremities with fine hairs. Length 0·77 mm. Setae of the posterior antennae reaching beyond the apices of the terminal claws. Caudal rami slender (fig. 37), their terminal claws simple, only slightly curved, the marginal seta short, attached near the distal end of the limb about one-fourth of its length from the apex. Sources A. D.

Cypridopsis circinata, n. sp. Plate XX, figs. 40, 41.

Shell very tumid, sub-spherical; seen from the side sub-ovate, gibbous, greatest height situated in the middle, and equal to about three-fourths of the length, extremities very broadly rounded, dorsal margin boldly arched, gibbous in the middle, ventral slightly convex (fig. 41); seen from above broadly elliptical, widest in the middle, width equal to two-thirds of the length, extremities evenly rounded off, lateral margins boldly and evenly arched, right valve slightly larger than the left (fig. 40). Caudal rami very small; only imperfectly seen. Shell-surface smooth, without sculpture of any kind, but densely clothed with short hairs. Length 0·65 mm. Dr. Dalziel's collection contained only one example of this species, taken in one of the two localities given for *Cypris subovata.*

CLADOCERA

Simocephalus, sp.

Diaphanosoma leuchtenbergianum? S. Fischer. Both of these from Sources A. B. C. D.

EXPLANATION OF PLATES

PLATE XVIII

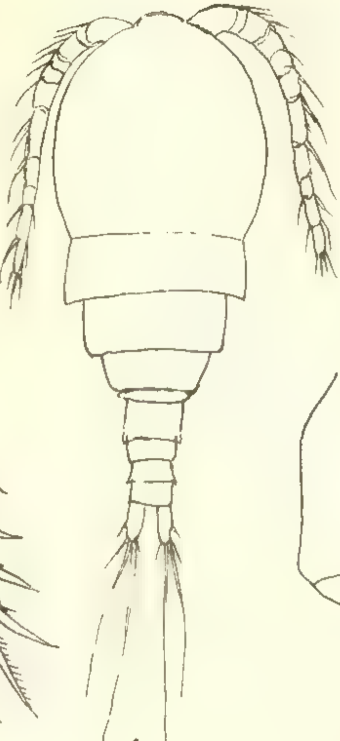
Cyclops nigeriae ♀

Fig. 1. Female seen dorsally. × 84.
Fig. 2. Foot of fifth pair. × 440.
Fig. 3. Urosome. × 160.
Fig. 4. Anterior antenna. × 160.
Fig. 5. Posterior footjaw. × 240.
Fig. 6. One of the swimming feet. × 180.
Fig. 7. Outer branch of third pair (?). × 180.

Cyclops virescens ♀

Fig. 8. Female seen ventrally. × 120.
Fig. 9. Anterior antenna. × 240.
Fig. 10. Urosome and last thoracic segment. × 240.
Fig. 11. Foot of first pair. × 320.
Fig. 12, 15, 16. Foot of second, third and fourth pairs. × 240.
Fig. 14. Foot of fifth pair. × 440.
Fig. 13. Last thoracic segment and first segment of urosome of male. × 240.

PLATE XVIII.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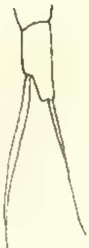
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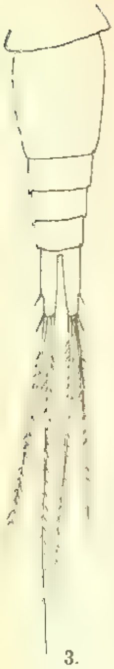
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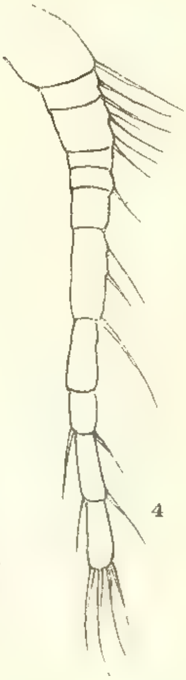
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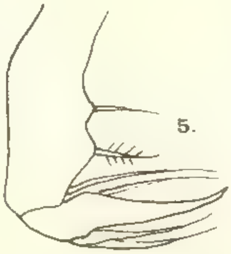
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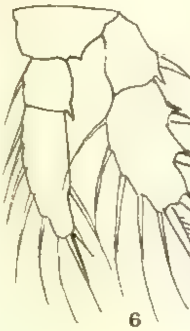
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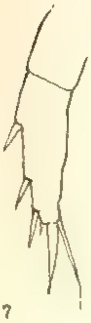
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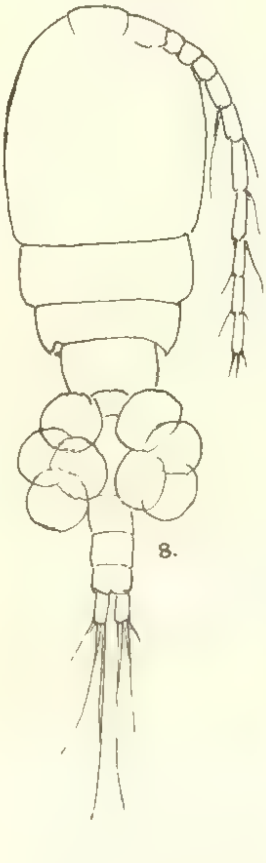
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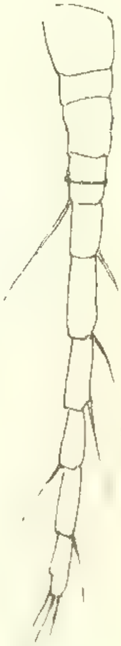
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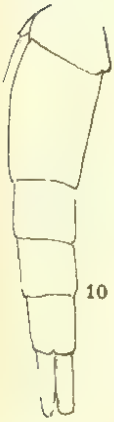
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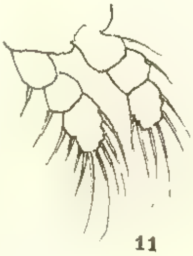
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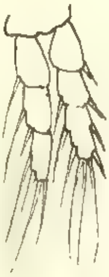
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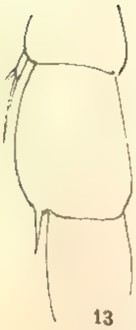
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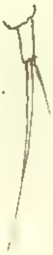
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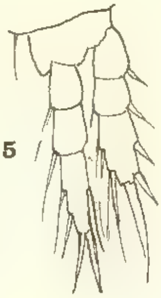
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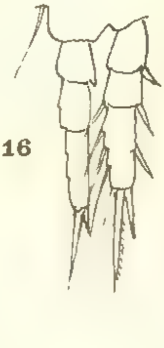
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G. S. BRADY del ad nat

Q

PLATE XIX

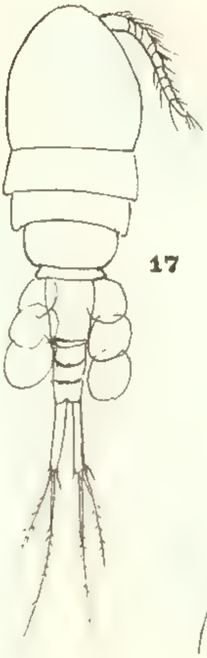
Cyclops longistylis ?

Fig. 17.	Female seen dorsally. × 84.
Fig. 18.	Urosome. × 180.
Fig. 19.	Foot of fourth pair, male. × 240.
Fig. 20.	Foot of first pair. × 240.
Fig. 21.	Posterior antenna. × 240.
Fig. 22.	Anterior antenna. × 240.
Fig. 23.	Last thoracic segment and fifth pair of feet. × 300.

Diaptomus nigerianus

Fig. 24.	Part of anterior antenna of male. × 140.
Fig. 25.	Urosome of female. × 140.
Fig. 26.	Posterior maxilliped. × 140.
Fig. 27.	Foot of fifth pair, female. × 180.
Fig. 28.	Last thoracic segment, female. × 140.
Fig. 29.	Female seen laterally. × 65.
Fig. 30.	Fifth pair of feet of male. × 140.

PLATE XIX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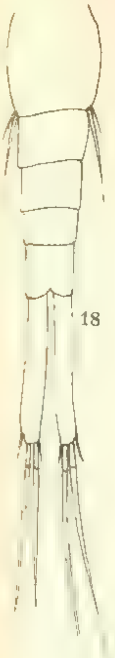
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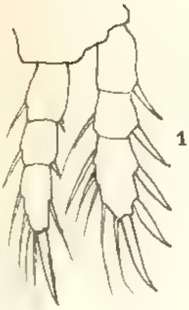
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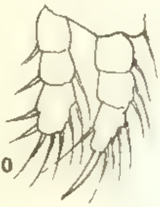
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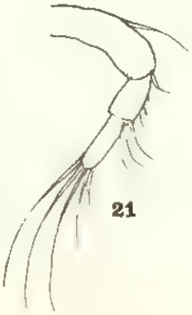
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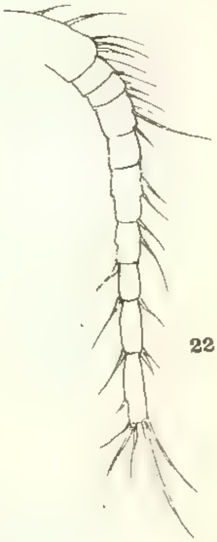
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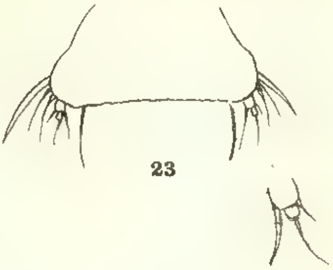
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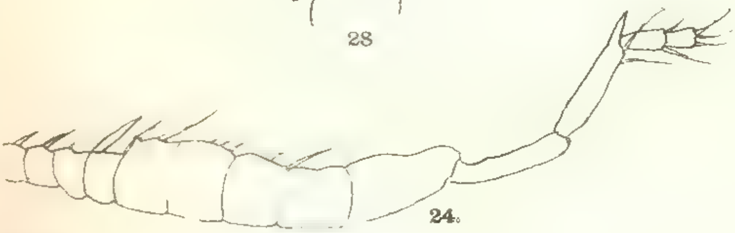
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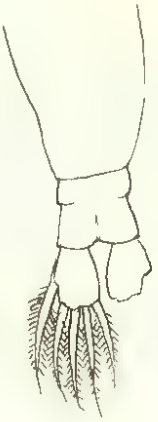
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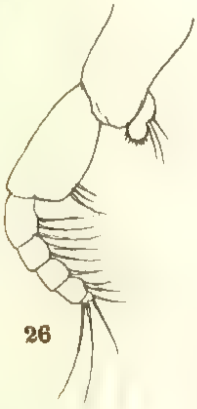
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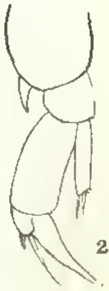
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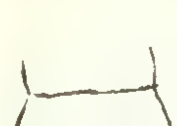
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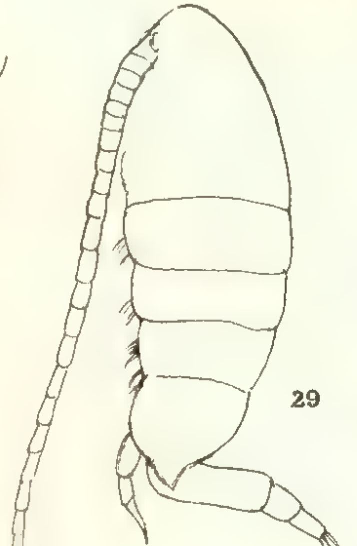
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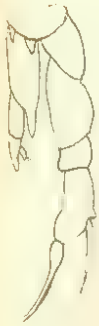
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G. S. BRADY del. ad nat.

PLATE XX

Cyclops brevipes ♀

Fig. 31. Female seen dorsally. × 100.
Fig. 32. Foot of first pair. × 350.
Fig. 33. Urosome. × 300.
Fig. 34. Anterior antenna. × 350.

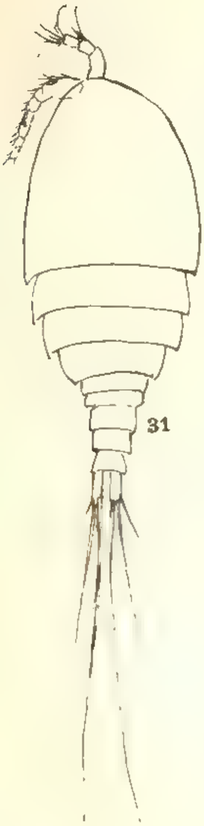
Cypris subovata

Fig. 35. Shell seen from left side. × 65.
Fig. 36. Shell seen from below. × 65.
Fig. 37. Caudal ramus. × 150.
Fig. 38. Extremity of foot of last pair. × 150
Fig. 39. Posterior maxilla. × 240.

Cypridopsis circinata

Fig. 40. Shell seen dorsally. × 84.
Fig. 41. Shell seen from right side. × 84.

PLATE XX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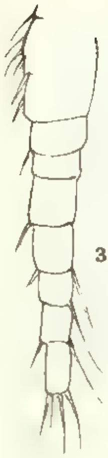
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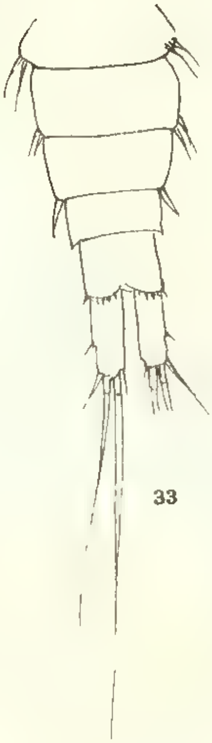
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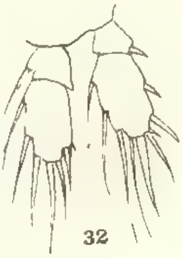
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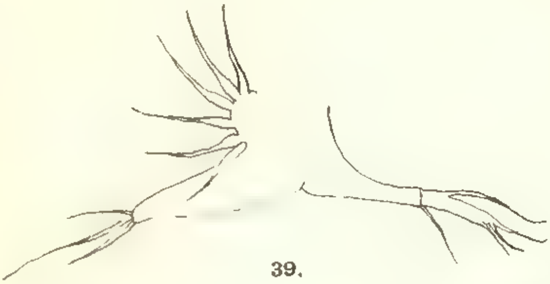
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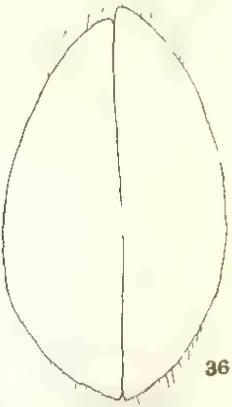
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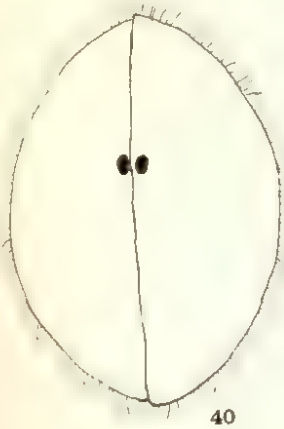
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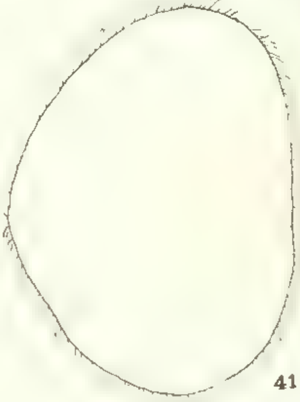
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G. S. BRADY *del ad nat.*

A NEW ANOPHELINE FROM THE FEDERATED MALAY STATES

BY

MALCOLM WATSON, M.D., D.P.H.,

CHIEF MEDICAL OFFICER, KLANG, FEDERATED MALAY STATES.

(Received for publication 21 June, 1910)

MYZOMYIA AURIROSTRIS, n. sp.

A small brown anopheline, with white apex to palpi—two broad white bands separated by slightly smaller black bands, and narrow white band near the base—legs unbanded. Proboscis banded. Wings with five costal spots and numerous other spots on the wing field.

Head ♀. Dark brown. Scales. Passing forward between the eyes there is a tuft of long white hairs (among which are to be seen narrow curved scales much as on the wings), and also about half a dozen long dark or brown hairs. Among the roots of these hairs, some white long narrow curved scales are seen. They do not extend either backwards or far from the middle line. Behind these are white upright shallow fork scales, and behind these again black fork scales. Four dark brown hairs project over the lateral region of the eyes.

Thorax. Brown. Dorsum brown, with long brown hairs; projecting over nape some narrow white curved scales. Elsewhere hairy.

Prothoracic lobes brown. Tuft of white narrow-curved and a few blunt scales projecting from anterior part. Elsewhere covered with hairs.

Abdomen. Brown, covered by numerous long brown hairs. Halteres golden scaled.

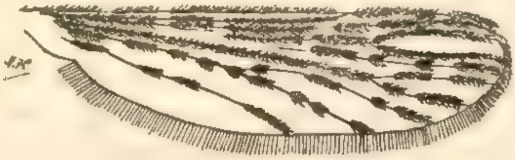
Legs. All brown scaled throughout; ungues, small, equal, simple.

Proboscis. Basal half black scaled. Apical half golden scaled except for a small but quite definite ring of black scales a shor distance from the apex. The apex is golden.*

Palpi. Black, with *four* white bands. The first band consist o a small but definite band of scales at the apex of the first joint. The apex of the last joint is white scaled, with a few white hairs projecting beyond the end. Then follow alternately a narrow black band, a broad white band, a slightly smaller black band, a broad white band, and the remainder of the palpi to the first joint black.

Antennae. Brown.

Scutellum. Brown, with a single row of long dark hairs, metanotum brown, nude.



Wing of *Myzomyia aurirostris*, n. sp.

Wings. Costa, black, broken by five gold spots—two in the basal half of the wing, of which only the outer involves the subcosta and first vein. In the outer half the spot at the junction of the costa and subcosta involves the first vein—the spot of which, however, begins almost where the costal spot ends, and extends towards the apex. It is equal in length to the costal spot. Equidistant between this and the apex is a spot involving the first vein, and at the junction of the first vein and costa there is also a golden spot involving the fringe and a small portion of the first vein and upper branch of second. The first vein, in addition to the above spots, has one just beyond the second costal spot. In one specimen a few golden scales are seen on the costa, not sufficient in numbers to constitute a definite spot, and only involving one side of the vein. On second long vein there are a few golden scales at the origin, a few at the fork, and a few half-way between these two. The third vein begins in a yellow spot, and the rest of the vein consists of four black and three yellow spots alternately—the black spot touching the fringe being longest of all. The fourth vein is black scaled to its fork, except for a few yellow scales opposite the origin of the third vein. At the fork there is a pronounced yellow spot, which extends down each branch, on each of which there is another small yellow spot. At the junction of the branches of the fourth and fifth veins, a few golden scales are seen. The fringe is black even at these points. The fifth vein throughout is alternately yellow and black. Between the origin and the fork there are three yellow spots; one yellow spot involves both branches, but mainly the upper; in addition, there are three yellow spots on each branch in addition to the yellow scales at the junction with the fringe. On first two-thirds of the sixth vein there are three yellow spots. The remainder of the vein is black scaled, with a few scattered golden scales about the middle. Except at the junction of the first vein, the fringe is black throughout. Wing scales long, narrow, lanceolate.

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*In the type specimen furnished by the author the integument at the tip is blackish in colour, and the few scales which remain are pale yellowish.—Ed.

Observations. Bred from two larvae, taken from a hole in the ground 1½ft. wide and 2ft. deep in the jungle next to Merton Estate, Klang, December, 1909.

Table Showing the Chief Differences between *Myzomyia albirostris*, *M. thorntonii*, and *M. aurirostris*

	M. albirostris	*M. thorntonii*	*M. aurirostris*
Palpi	Two broad apical bands and one narrow basal one	Three broad apical bands and a narrow basal band	As in *M. thorntonii*
Proboscis	Apical half pale scaled	Apical half light scaled with a narrow brown band near the apex	As in *M. thorntonii*
Wing Fringe	Pale spots at each vein junction	Mottled—the light spots occurring for the most part at the apices of the veins, long and short scales to the 5th and 6th distinctly yellow	Black
Legs	Unspotted, brown	Spotted and banded	Unspotted, brown
Prothoracic lobes ...	—	Brown flat scales	Tuft of white narrow curved scales from anterior part—elsewhere hairs

ON THE OCCURRENCE OF SCHIZOGONY IN AN AVIAN LEUCOCYTOZOÖN, *L. LOVATI*, PARASITIC IN THE RED GROUSE, *LAGOPUS SCOTICUS*

BY

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Plate XXI

Although the Parasitic Protoza are much studied at present, there is no doubt that more facts are required about the life-cycle of many parasites of whose history certain stages are well known. Among such parasites are the avian *Leucocytozoa*.

During the past two years I have spent some of my time as Protozoologist to the Grouse Disease Inquiry, in the investigation of the Protozoa parasitic in the grouse. One, at least, of the protozoal parasites of the grouse, *Eimeria (Coccidium) avium*, is the cause of a rapid and fatal disease among grouse chicks. Among the parasites present in some apparently healthy grouse was a Leucocytozoön, *L. lovati*, first recorded by Seligmann and Sambon in a short note in 1907. My investigation of *L. lovati* led to the elucidation of another phase (schizogony) in its life history.

The host cells of the avian *Leucocytozoa* may be either mononuclear leucocytes or erythroblasts, or both. The exact nature of the host cell of *Leucocytozoa*, which is somewhat controversial, will not be discussed in this paper. Concerning the *Leucocytozoa* themselves, gametocytes, both male and female, have been described from the blood of the host. The male gametocyte of *L. lovati* (Pl. XXI, fig. 1) possesses hyaline, pale-staining cytoplasm, and a rather large nucleus containing a number of chromatin granules. The female gametocyte (fig. 2) has a deeply staining granular and somewhat alveolar cytoplasm, with a central nucleus containing a karyosome. The exact delimitation of the nucleus of a *Leucocytozoön* within its host cell is often difficult.

In the specimens of *L. lovati* that I examined, the microgametocytes were found to measure from 13μ to 17μ by 6μ to 12μ while the macrogametocytes were 14μ to 20μ by 10μ to 16μ.

Till recently, the gametocytes and gametes only of avian *Leucocytozoa* were known. However, parasites somewhat intermediate in type between the male and female gametocytes occur, though such intermediate forms are not at all common in the peripheral blood of the host. The intermediate forms in the case of *L. lovati* may be seen in the blood of the spleen of the grouse, though they may be easily overlooked.

In the summer of 1909, I examined two fresh grouse in whose peripheral and heart blood living *Leucocytozoa* were seen. The internal organs were immediately examined after the death of the birds. In stained preparations of the spleens of the two birds, small vermicular forms were encountered, both free and just entered into their host cells. Careful search showed that in the spleen were many full-grown *Leucocytozoa*, some of which exhibited no very definite sexual differentiation. While the gametocytes encountered in the blood often produced marked elongation of the host cells, the deformation in the case of the special splenic forms was slight (fig 3), and the host cells seemed almost entirely absorbed (fig. 4). These parasites are the schizonts, which are slightly smaller than the gametocytes, and measure 11μ to 14μ by 8μ to 11μ. The cytoplasm of the oval schizonts becomes somewhat concentrated while they are uninucleate (fig. 4). The character of the nucleus of the schizont approaches that of the microgametocyte, while the general cytoplasm resembles that of the macrogametocyte, but differs from it in being less alveolar and possessing smaller granules.

Nuclear multiplication occurs, apparently, by a series of rapid binary fissions, and the daughter nuclei, some of which may remain united (fig. 5) for a short time, ultimately migrate towards the periphery. Segmentation of the cytoplasm around the small nuclei occurs (fig. 6), and the result is that some twelve to twenty merozoites (fig. 7 are produced. Each merozoite is a small, vermicular, somewhat curved organism, capable of active movement. The merozoites measure 7μ to 8μ by 1μ to 1·5μ. Some residual protoplasm is found in the remains of the schizont after the merozoite formation is completed. The merozoites escape (fig. 8) from the tenuous envelope of the schizont and become free-living forms (fig. 9) in the blood of the spleen. But their free stage of existence is of short duration. Leucocytes or immature erythrocytes are encountered, and the young merozoites penetrate the host cells and enter upon the trophic phase of their existence, ultimately differentiating either as sexual individuals or as schizonts.

The occurrence of schizogony in *L. lovati* is very difficult to demonstrate. Though many infected birds may be examined, schizogony may not be detected, for the period of schizogony may not be attained, and also this form of multiplication is passed through with remarkable rapidity, so that merozoites may be formed and dispersed before the investigator has had time to examine the material available. Rapidity of schizogony is also found in such parasites as *Coccidia*, where often it is not easy to obtain preparations exhibiting schizogony, while some gametocytes can usually be obtained in any preparation from an infected host. In connection with schizogony of *Leucocytozoa*, great interest attaches to the discovery of Mathis and Léger of periodic increases in the number of the gametocytes of *L. caulleryi* in the peripheral blood of its host, the domestic fowl of Tonkin, for which increase no explanation has hitherto been afforded. It seems to me that during the intervals between successive crops of parasites, asexual multiplication probably occurs. As in *L. lovati* schizogony occurs in an internal organ, namely, the spleen, it is suggestive that a similar condition prevails in *L. caulleryi*; and as in other Protoza, the schizogony culminates in sexual differentiation, so in *L. caulleryi* it seems likely that a similar sequence may occur.

It may be added that preparations of bone marrow of the infected birds did not furnish schizonts, though it is possible that schizogony might be found to occur therein, if abundance of material were available for research. *L. lovati* did not appear to be very harmful to the grouse investigated, and in the two cases in which schizogony occurred, no other protozoan parasite was present in the blood of the grouse, all of whose organs were minutely examined.

L. lovati may be transmitted from grouse to grouse by the agency of the grouse-fly, *Ornithomyia lagopodis*, for vermicules devoid of melanin pigment have been found in the gut of the fly. But, as with most blood parasites, further work is necessary with regard to the exact mode of transmission of protozoal parasites from host to host.

The avian *Leucocytozoa*, as exemplified by *L. lovati*, in which schizogony is now shown to occur, are, then, typical members of the *Haemosporidia*, allied to the malarial parasites.

In conclusion, while the method of schizogony in *L. lovati* is as indicated in this paper, it does not follow that the multiplicative processes of all avian *Leucocytozoa* are on the same lines, though I think that it is probable that in all these avian blood parasites, the period of schizogony is a short one, and that much patient investigation under the most varied conditions will be necessary to establish schizogony in most members of the avian *Leucocytozoa*.

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EXPLANATION OF PLATE XXI

The figures are slightly diagrammatic. Magnification of figs. 1 and 2 about 1,800 diameters; of figs. 3 to 9 about 2,000 diameters.

Figs. 1 and 2. Diagrams of male and female gametocytes of *Leucytozoön lovati.* The male gametocyte (fig. 1) is usually slightly smaller than the female (fig. 2), and its cytoplasm is hyaline. Chromatoid granules often occur at the ends of the oval or round parasites. The host cell has become spindle-shaped in each case, with its nucleus pushed to one side by the parasite.

Figs. 3 to 9. Diagrams of schizogony of *Leucocytozoon lovati,* as seen in smears of the spleen of the avian host.

Fig. 3. Schizont, with remains of the host cell at either pole and host-cell nucleus to one side.

Fig. 4. Uninucleate schizont, with host cell almost entirely absorbed except for slight remains of the host-cell nucleus to one side.

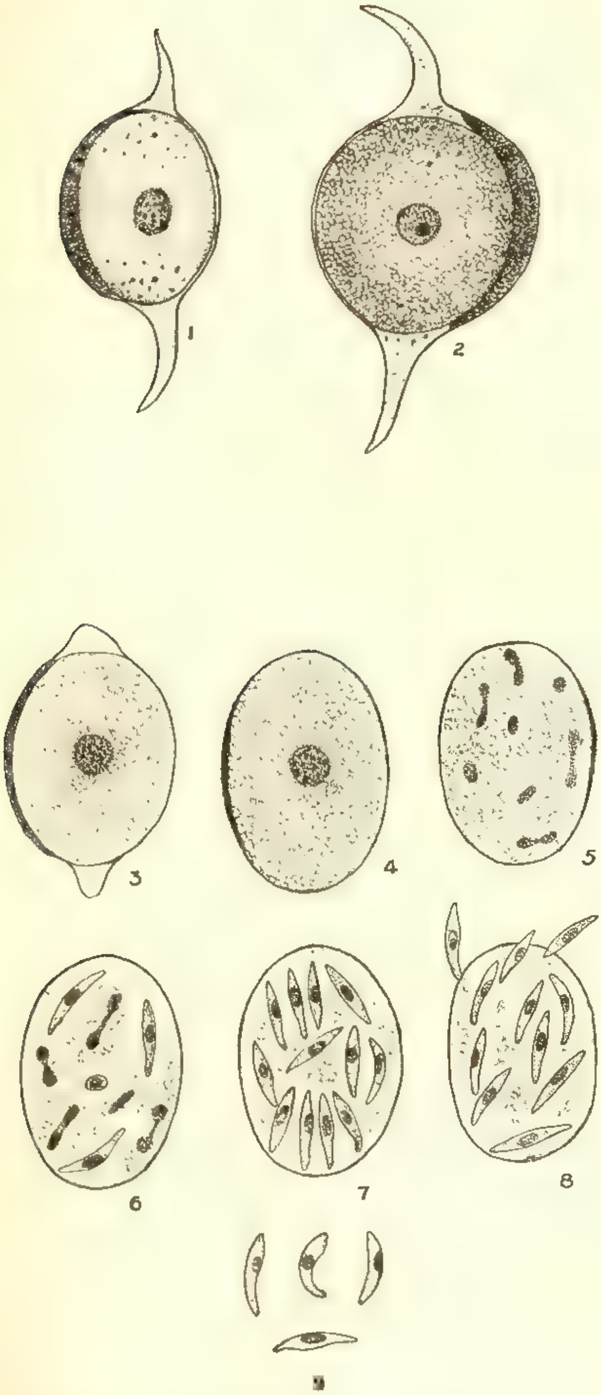
Fig. 5. Schizont, showing nuclear multiplication. Very slight remains of host-cell nucleus on the right side.

Fig. 6. Schizont in process of formation of merozoites.

Fig. 7. Schizont showing merozoites differentiated within.

Fig. 8. Merozoites beginning to escape from schizont.

Fig. 9. Group of free merozoites.



SCHIZOGONY OF LEUCOCYTOZOON LOVATI.

P Press, lith.

[H. B. Fantham, del.

A CASE OF SLEEPING SICKNESS STUDIED BY PRECISE ENUMERATIVE METHODS: REGULAR PERIODICAL INCREASE OF THE PARASITES DISCLOSED

BY
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AND
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(Read before the Royal Society, 16 June, 1910)

(Received for publication 5 July, 1910)

PREFATORY NOTE BY R. ROSS

For a long time it has appeared to me that much light might be thrown on infectious diseases, immunity, and treatment, by more exact enumeration of the infecting organisms, and that we might even be able ultimately to apply mathematical reasoning to the study of these subjects. In 1903* I elaborated a method of blood examination, called the thick-film process, which enables us to detect small organisms in the blood about twenty times more easily than in ordinary preparations; but for the lack of the necessary assistance I was long unable to apply the method to the laborious enumeration of such organisms. Recently, however, the Advisory Committee for the Tropical Diseases Research Fund has placed considerable funds at the disposal of the Liverpool School of Tropical Medicine for the study of cases in the tropical ward at the Royal Southern Hospital, Liverpool, with the result that the investigations referred to were commenced by Dr. David Thomson and myself from the beginning of this year. As I expected, methodical counting of the parasites has at once verified or disclosed several facts of importance in connection with malaria and trypanosomiasis. We now limit ourselves to a brief description of the remarkable periodical increase of *Trypanosoma*

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* Lancet, 10th Jan., 1903, and Thompson-Yates Reports, Vol. V, part I, 1903.

gambiense revealed by careful daily counting in a case in my clin (the case, which is still under treatment, will be fully reporte elsewhere).

W. A., male, aged 26, a strong young man, born in Northumberland, was infected in North-East Rhodesia in September, 1909, the trypanosomes being found in his blood Africa on November 17. On coming to Liverpool for treatment he was admitted into the Southern Hospital on December 4. From then until February 16 (seventy-three days) the number of trypanosomes in his blood was estimated only by the rough methods in common use—that is, by the proportion of trypanosomes to red cells, or leucocytes, or to 'fields' of the microscope examined. These methods are obviously open to such great error that they can scarcely be depended upon to indicate any but very large differences in the numbers of objects counted. During the seventy-three days forty-six counts were made, but on several occasions none was attempted for three or four days in succession so that, even if the methods of counting employed had been more accurate, sudden fluctuations might easily have been missed. Hence, as was to be expected, the graph during this period is very irregular and almost worthless. On admission on December 4 the patient was reported to contain about 6,000 trypanosomes per cubic millimetre of blood, and large numbers, amounting to about 3,000 per cubic millimetre, were found on December 17 and 28, and on January 16. All this time the patient was given the usual treatment with atoxyl and mercury, and received altogether ten doses of 2 to 4 gr. of the former. Nevertheless, the parasites never fell below about 200 per cubic millimetre in number, as roughly estimated.

It was then found, however, that atoxyl was injuring the patient's sight—as sometimes happens—and other treatment was substituted. At the same time we elaborated a much more correct method of counting all the parasites in measured quantities ($\frac{1}{4}$ to 1 c.mm.) of blood taken in thick film, and from February 16 onward the patient's trypanosomes were estimated daily by this method by one of us (D.T.). The attached chart gives the remarkable graph obtained up to the present (April 30).

The numbers of trypanosomes found were scrupulously recorded, and the smoothness and regularity of the graph suggest that there was no very great error of observation. The blood was taken every day at about 10 a.m., but on April 5 and 6 several counts were made daily.

It will be seen that between February 16 and April 30 (seventy-three days) there were eleven rises in the number of the parasites. Up to April 7 there were seven rises, at intervals of seven or eight days. During this period the patient was given no atoxyl, but was treated with large doses of quinine (30 to 40 gr.) daily, with frequent doses of methylene blue and with trypan red on March 17, 18, 19, and 20.

On April 5 it was decided to administer atoxyl again, as shown on the chart, together with mercury and other treatment.

The temperatures were taken by the sister of the ward, and it will be seen that there has always been a tendency to a slight rise in temperature concurrent with the rise in the number of parasites—the two curves thus confirming each other. Only the maximum and minimum temperatures are entered in the accompanying chart.

The great regularity of the rises can scarcely be compatible with a mere chance distribution. It will also be observed that the rises were of two kinds—namely, high rises and low rises—and that the two kinds alternated with regular periodicity until April 18, at which point the cycle appears to have become distorted—probably in consequence of the treatment. The regularity of alternation of the high and low rises is so well marked as to recall the picture of a double tertian malaria, and to suggest that two independent sets of parasites may exist in the patient, just as often happens in malaria.

The large dose of atoxyl given on April 5 seems to have had no effect whatever on the following rise; but the succeeding rises were apparently modified for some reason. The value of the enumerative method for therapeutical research is obvious.

Of course, many other facts in connection with the case have been recorded, and parallel work is being done on sub-inoculated animals and on the parasites. It is therefore inadvisable to attempt at present any discussion of the many interesting theoretical questions which arise.

We are much indebted to the Director of the Sleeping Sickness Bureau (Dr. Bagshawe) for having given us references to the literature on the subject of such fluctuations. In the original case of Dutton and Ford it was noted that the parasites varied in numbers, and that a parallel rise in the patient's temperature occurred. Manson and Daniels[*] chart the number of parasites compared with 500 leucocytes; but the error of this method is very large and their graph is quite irregular. They abandoned counts in measured quantities of blood as 'unreliable.' Thomas and Breinl[†] showed that in three cases of sleeping sickness the numbers of trypanosomes found in 'fresh cover-slip preparations' varied irregularly from time to time. Koch, Beck, and Kleine (1909) remark on the irregularity of the appearance of *T. gambiense* in African natives, and state the parasites are present for two to five days and absent for two to three weeks. Salvin-Moore and Breinl[‡] show a graph with two undulations and a final premortal rise in two heavily infected rats, and give a detailed description of corresponding changes in the parasites. Apparently, hitherto, only irregular variations in the numbers of the parasites seem to have been recognised; probably the large error due to inadequate methods of counting has disguised the regular periodicity of the variation shown by more exact counts in the eleven successive undulations observed in our case.

We should add that our methods enable us to detect parasites when they are in numbers so small that their detection by the ordinary methods would be exceedingly laborious. Hence if our case had been studied by the ordinary methods, probably only the crests of the rises would have been visible in the chart, and it would have been said that the parasites had disappeared in the intervals.

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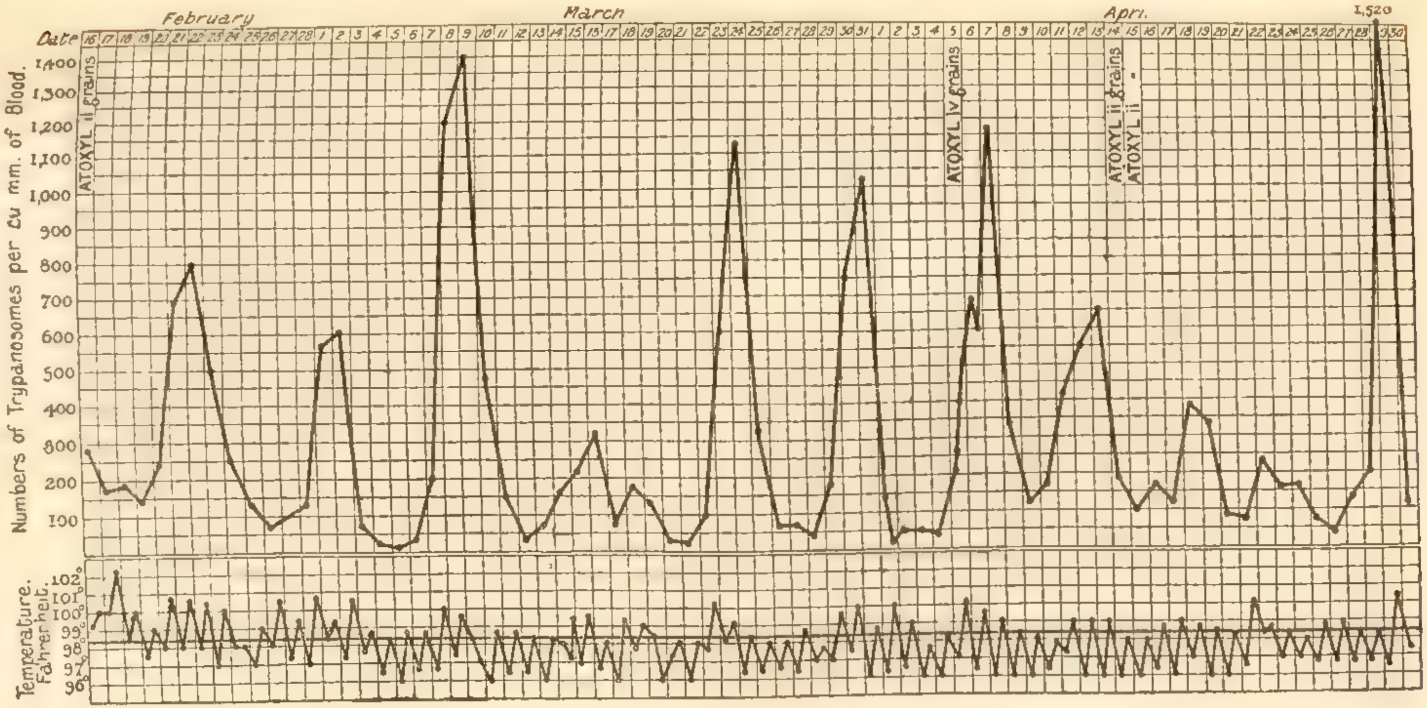
[*] Brit. Med. Journ. May 30, 1903.

[†] Memoirs of the Liverpool School of Trop. Med. Vol. XVI, 1905.

[‡] Annals of Trop. Med. and Parasit. Vol. I, No. 3, 1907.


February
March
April
1,520
Date
16 17 18 19 20 21 22 23 24 25 26 27 28 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
ATOXYL ii grains
ATOXYL iv grains
ATOXYL ii grains
ATOXYL ii "
Numbers of Trypanosomes per cu mm. of Blood.
1,400
1,300
1,200
1,100
1,000
900
800
700
600
500
400
300
200
100
Temperature. Fahrenheit.
102°
101°
100°
99°
98°
97°
96°

SOME ENUMERATIVE STUDIES ON MALARIAL FEVER

BY

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AND

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(*Received for publication* 15 *November*, 1910)*

1. *Preliminary.* For many years past little information which is both new and exact has been added to our knowledge of the pathology of malaria. This has probably been due to the exhaustion of the older methods of research which, being purely qualitative, have failed to indicate the precise correlations between the numbers of the parasites present in a patient and the various pathological and therapeutical reactions. For example, out of fifty-one and thirty-eight successful inoculations of men by means of infected blood and infected Anophelines respectively, in not a single one has any exact estimate been given of the number of parasites inoculated or recovered after the lapse of the incubation period; and, though many researches on quinine have been made, we know of none in which its direct effect upon the numbers of the parasites in the patient has been correctly measured. Moreover, the older methods often failed to reveal the parasites at all, unless they were present in large numbers. Hence our first care was to elaborate more exact methods both for detection and for enumeration. This was done early in the year (by R.R. and D.T.), though we have by no means reached finality yet. The new methods were next employed for all the cases in the Tropical Ward, daily counts of the parasites and often of the leucocytes, together with other estimations, being made (by D.T.). At the same time parallel chemical studies were carried out and therapeutical ones, and the measurements were carefully charted and compared. Even at this preliminary stage the results (which are given at the end of this paper) include more accurate verifications of some old conjectures, and demonstrations of some new theorems.

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*(Read before the Royal Society, Dec. 8th, and reprinted from Proc. Roy. Soc., Series B., Vol. LXXXIII, p. 159, with Addenda.)

2. *The detective method used.* In the ordinary methods the blood is spread out in a thin film, liquid or dry, one cubic millimetre covering say four square centimetres of area. If the field of the oil-immersion lens has a diameter of 0·165 mm. and can be thoroughly searched by the eye in three seconds, about twelve hours' work would be required to examine the whole of 1 c.mm. of blood in this way; and if the specimen contains only one parasite it may not, by bad luck, be found until nearly the whole of the area has been searched. We have therefore employed the 'thick film process' described by one of us (R.R.) in 1903. In this 1 c.mm. of blood is spread out over only about one-quarter of a square centimetre or less, and dried; the haemoglobin is then gently washed out with water; and the residue, consisting of parasites, leucocytes, platelets, and the stromata of the red corpuscles, is carefully stained by any convenient method, with or without fixation. By this simple means the whole of a cubic millimetre of blood can be searched in less than an hour; but the identification of the Plasmodia requires considerable practice.

3. *The enumerative method used.* The younger *Plasmodia* are too small to be counted by the use of an ordinary haemocytometer; hence their number has usually been estimated by comparison with the number of red or white cells, the latter figure being determined by the haemocytometer. This gives a large compound error; for if e is the percentage error made in counting the red or white cells in one specimen, and e^1 is the percentage error made in computing the proportion of parasites to each corpuscle in that specimen, then, by a simple calculation, the total percentage error will be $e + e^1 + ee^1/100$. (For example, if $e = +5\%$ and $e^1 = +10\%$, the total percentage error will be $+15·5\%$.)

Our method consists simply in making a measured quantity of blood into a thick film preparation and then counting *all* the parasites in it. The blood is measured by determining the diameter of a fine capillary tube under the microscope, and then calculating and marking off the length of the tube required to hold a given quantity (as suggested by Dr. Wakelin Barratt). Thus a uniform tube of 0·180 mm. diameter and 4 cm. length will contain 1·018 c.mm.—a convenient size. The blood is sucked into the tube and then quickly discharged upon the glass-slide in one or several measured droplets, first samples being rejected. The dehaemoglobinized thick film is fixed before staining in order to avoid loss, and the whole area is scrupulously searched with the aid of a sliding stage.

Mr. M. Greenwood, Junr., informs me that if n is the number of parasites counted in the unit (say 1 c.mm.) of blood, and m is the number of such units in the patient's whole body, then the total number of parasites in him, assuming uniform distribution, will be $mn \pm 0{\cdot}67449 m \sqrt{n}$. Thus the probable percentage error is $67{\cdot}449 / \sqrt{n}$, the factor m cancelling out from the ratio. This is important, because it shows that the error depends, not on the magnitude of the measured sample taken, but upon the number of parasites actually counted in it. Hence, in order to obtain results within an assigned error, we take a large sample when the parasites are scarce and a small one when they are numerous; the number of parasites which we must find and count being given by the formula $n = 4550/e^2$, where e is the permissible error.

The greatest error occurs when the parasites are so scarce that it is difficult to find enough of them to count up to the required standard; or when they are so numerous that it is difficult to measure a volume of blood small enough to contain an easily countable number of them. In these preliminary researches we have seldom examined at one sitting more than 1 c.mm. of blood, or less than ¼ c.mm.

When the parasites were very numerous we have sometimes fallen back on estimating their number by comparison with leucocytes, but are now elaborating improvements and special instruments for such, and other, details. The method is also accurate and rapid for other blood parasites, and for leucocytes. Other sources of error are (1) inaccurate measurements of the quantity of blood used, and (2) difficulty of seeing or distinguishing badly stained parasites.

One kilogram of blood of average density (1057·5) contains 945,626 cubic millimetres, and the blood in a man's body is estimated to weigh about 4·9 per cent. of the total body weight, from which data it is easy to compute the total number of parasites in a patient. A man of 64·74 kilograms, or about ten stone, will contain about 3,000,000 c.mm. of blood.

4. *The cases studied* by us numbered thirty-three. All had been infected in West Africa or America, so that none was of less than some weeks' duration when admitted into hospital in Liverpool. All were males of from eighteen to sixty years of age. They were mostly shipmen or traders, and two were negroes. The parasites of all were counted almost every day, and sometimes several times a day; and the temperatures were taken every four hours, or more often. One case showed *Plasmodium malariae*, together with crescents (sexual forms of *P. falciparum*). Eight showed *P. vivax* only, and twenty-four *P. falciparum* only, two of the latter containing only sexual forms. Quinine was often withheld for some days while other methods of treatment were used. The cases were studied for 600 days altogether. There were no deaths.

All the cases are tabulated at the end of the paper for verification of details, and charts of cases 17 and 20 are given. At this preliminary stage of the enquiry we have undertaken to study only the gross daily correlations between the numbers of parasites and other phenomena, leaving more minute hourly and four-hourly analysis to future work. Hence if a number of counts or other observations have been made on one day we have recorded only the averages in the tables. For temperatures, however, it has been thought best to record maxima instead of averages, and, as the tables should be as economical as possible, we have used the *haematothermic scale* suggested by one of us (R.R.), in which the ten degrees between 95° and 105° F., or between 35° and 45° C., are divided into 100 parts. Except Case 6, specially studied by Dr. Fantham, we have not distinguished in the tables the various forms, sets or stages of *P. vivax*, this being reserved for future study. Similarly, the treatment, leucocytes, haemoglobin and urobilin are not differentiated too minutely. Case 25 was counted by Dr. Korke.

5. *The correlation between the parasites and the fever.* It is well known that fever is caused only by the asexual forms of the parasites; but though these are generally thought to be more abundant during pyrexial periods, no extensive numerical studies on the point appear to have been made, with the result that some sceptics still profess doubts on the subject. Our cases, except four without fever (Cases 1, 15, 16, 27), would seem to

indicate a very strong, almost convincing, correlation. Twenty-one of them suffered each from one pyrexial period, lasting from one to seven days and preceded or followed by apyrexia; and in all except Case 32, the asexual parasites were very much more numerous during the pyrexial period. Nine of the cases (7, 17, 18, 19, 20, 23, 24, 26, 32) suffered from one or two relapses each (thirteen relapses altogether); and every relapse, as well as every original attack, was associated with a comparatively large number of parasites (forty-three pyrexial periods altogether). Similarly, there were forty-six apyrexial periods (including the four cases without fever), all associated with comparatively small numbers of asexual parasites; and conversely not a single marked rise in the number of asexual parasites took place without corresponding fever.

For further details we compare the numbers of parasites per c.mm. counted on days of fever with those counted on days without fever. A febrile day is taken as any one on which the patients' temperature exceeds 98·6° F. (= 36° Haematothermic Scale), or any one which comes between two tertian paroxysms (Cases 9, 14, 24). The non-febrile days on which no asexual forms could be found in 1 c.mm. of blood are omitted. The results are:—

	Cases	Days	Total parasites	Average per day	Ratio
P. vivax—					
Fever	8	30	136,062	4,535	36·3
No fever	8	68	8,487	125	—
P. falciparum—					
Fever	21	97	1,319,880	13,607	29·6
No fever	19	83	38,277	461	—

If we had not omitted the non-febrile days on which no parasites were found, the non-febrile daily averages would have been much smaller than the figures given above, and the ratio of the febrile averages to them much larger; so that the febrile excess is very marked. An examination of the details will further convince the reader of the great fall in temperature which accompanies the fall in the number of parasites. We should note that the comparatively small numbers of *P. vivax* found are due to the fact that the specimens generally contained a large proportion of mature parasites, not as yet disintegrated into spores—these, of course, being absent from peripheral blood containing *P. falciparum*, in which only spores or young forms generally occur. There is no reason for considering quinine in this connection, because it probably affects the fever only through its action on the parasites.

6. *The degree of fever associated with various numbers of parasites.* We know of no extensive and exact studies on this point, and ours are not yet complete. On forty-seven occasions the number of parasites was carefully compared with the maximum degree of temperature reached during the febrile paroxysm caused by them, and the following results were obtained:—

P. vivax **(12 occasions)—**					
No. of parasites	1,500	1,400	580	440	430
Maximum temp. °H.F. * ...	84	101	97	102	76
No. of parasites	260	256	232	150	133
Maximum temp. H.F.	76	54	46	44	54
No. of parasites	100	83			
Maximum temp. H.F.	34	20 (rigor)			
Co-efficient of correlation *r* = 0·68561 ± 0·10318					
P. falciparum **(35 occasions)—**					
No. of parasites	303,000	58,000	55,000	54,000	50,000
Maximum temp. °H.F.	108	82	88	100	98
No. of parasites	45,000	36,000	34,000	31,000	26,000
Maximum temp. H.F.	76	60	86	76	94
No. of parasites	26,000	25,000	16,000	15,000	15,000
Maximum temp. H.F.	101	80	52	54	54
No. of parasites	15,000	9,700	9,000	7,500	7,400
Maximum temp. H.F.	70	56	70	90	40
No. of parasites	6,700	6,500	6,000	5,800	5,200
Maximum temp. H.F.	86	83	52	70	76
No. of parasites	4,000	4,000	3,600	3,000	2,600
Maximum temp. H.F.	70	40	80	38	64
No. of parasites	2,500	2,000	1,860	1,000	200
Maximum temp. H.F.	50	46	38	15	50
Co-efficient of correlation *r* = 0·60331 ± 0·07251					

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* H.F. = Haematothermic scale Fahrenheit.

These figures, together with the numerous ones given in the table of cases, show a very marked correlation, but there are, of course, many minor deviations. In numbers of the specimens there were certainly two sets of parasites of different ages, which were often counted together, though only one set could have been concerned with the fever. It is probable also, though by no means certain, that the resistance to the toxin of the *Plasmodia* varies, not only in different persons, but in the same person at different stages in the course of his infection and under different physiological conditions. Our new methods will, we hope, enable us shortly to undertake very detailed studies on these points.

7. *The pyrogenic limit.* It is quite evident that if the parasites fall below a certain limit they are no longer numerous enough to cause fever. Thus with *P. vivax*, an average of 125 parasites of all ages and forms counted during sixty-eight days in eight cases, and with *P. falciparum* an average of 460 asexual forms counted during eighty-three day in nineteen cases, failed to produce fever. The actual limit above which they become pyrogenic probably varies in different cases. With *P. vivax* as many as 1,500, 852 and 540 were found in Cases 2, 6, and 7 without fever, and as few as 150 and 50 in Cases 5 and 9 with very slight fever (98·6° F.), so that about 200 to 500 may perhaps be taken as the usual limit. With *P. falciparum*, as many as 1,620 (asexual) were found as an average of four non-febrile days in Case 26, and 1,196 as an average of six non-febrile days in Case 25; while in Case 18, from 16 to 1,860 were present for twenty-two days without causing any marked fever, so that 600 to 1,500 may perhaps be adopted as the usual limit. With *P. malariae* (Case 1), an average of 79 were present for thirteen days without fever, but about 140 twice caused slight rigor.

8. *The asexual forms between the relapses.* It is generally thought and taught that these forms tend to 'disappear' between the relapses—though of course exceptions, especially in children, are noted—and to reappear during them; and several hypotheses, such as that of parthenogenesis (F. Schaudinn) have been advanced explain the supposed phenomenon and have led to what appears to be much waste of work. We find that the asexual forms do not

necessarily disappear at all between the relapses, but are generally still to be found in small numbers per c.mm. on most of the days. The apyretic periods preceding the thirteen relapses in our cases (7, 17, 18, 19, 20, 23, 24, 26, 32) lasted 114 days altogether (nearly nine days each on the average). On eighteen of these days no observations were made; but on the remaining ninety-six days the parasites were found in small numbers in 59, or 61 per cent., and were not found only in 37. Moreover, the general trend of the curves suggests that they were not found on these days only because their numbers were a little too few for detection. The parasite curve, at its height during a pyrexial period, generally falls very rapidly at first and more slowly later, and tends to reach its lowest about half way between two apyrexial periods. At this point it may or may not remain above the detectable limit (by thick film methods). After this it was observed (by D.T.), especially in Cases 7, 17, 23 and 24, to begin mounting slowly at first, until when it reached the pyrogenic limit, another pyrexial period commenced. All this is scarcely compatible with the speculation that the apyrexial periods are due to the abrupt death of most of the asexual *Plasmodia*, or to their conversion into 'resting stages,' etc. Nor do such speculations appear to be at all necessary. It is easy to see that the survival of comparatively small numbers of the asexual forms will suffice to keep the infection alive, not only for the short periods observed by us, but for 'relapses of long interval,' and for months or years. Obviously, if only a few parasites per c.mm. are present, they may easily be overlooked in the small amount of blood (say 0·1 c.mm.) usually examined by thin film, and may then be reported as being absent: yet many millions may still exist in a patient who contains say 3,000,000 c.mm. of blood. The speculation regarding parthenogenesis in malaria and the case of Schaudinn, supposed to support it, have been criticised elsewhere by one of us (R.R.).[*] In two of our relapsing cases (19, 26) no sexual forms at all were found during thirty-five different thick film examinations. Even if they were present in small numbers (crescents do not collect in the spleen), yet the numbers of asexual parasites found in or just before the relapses (24 and 400 per c.mm.) cannot be explained by parthenogenesis unless we suppose that each sexual form produced by 'gametoschizogony' 840 or 14,000 spores. Until better evidence for such views is adduced, our results justify the doctrine that the malarial infection is kept continuously alive simply by the persistence of the asexual form in varying numbers; and that fever occurs only when the forms are numerous enough to produce it.

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[*] Prevention of Malaria (Murray, 1910).

9. *The effect of quinine on the asexual forms.* The destructive effect of the drug is of course everywhere recognised, though very little completely satisfactory statistical evidence can be cited in proof. Many laborious researches have been made regarding the comparative utility of the various salts, but these have been confined almost entirely to estimating the rate and percentage of absorption, judged from urinary elimination. The subject is of the greatest sanitary and medical importance; but, so far as we can see, it can be usefully studied only by the more detailed enumerative analysis which we propose soon to undertake. Our daily analysis gives little more light; but the following figures may be mentioned. Cases 15, 16 showed no asexual forms; Cases 11 and 13 were so severe that quinine was given at once; Cases 1, 5, 27, 31 and 32 were so mild that quinine was withheld during the period of observation; and in the remaining cases the drug was withheld only for some days (except an occasional necessary dose) and was then given continuously while the parasites were being counted. We have therefore added together all the daily counts during the periods of no-quinine and quinine respectively. With eight cases of *P. vivax* there were forty-five no-quinine days showing 104,032 total parasites, or an average of 2,312 a day; and there were 74 quinine days with 31,769 total parasites, or an average of only 429 a day (one-fifth). With *P. falciparum* (19 cases), 147 no-quinine days showed an average of 31,136 asexual forms, and 148 quinine days an average of 1,535 (one-half). We should have expected that the proportion of quinine-day parasites would have been smaller; but the no-quinine counts are diminished by the inclusion of the five mild cases and also by doses of 10 to 20 grains which were given on ten isolated occasions. On quinine days the doses are generally 20 to 30 grains a day, and Cases 11, 13, 14, 15 and 16 and parts of other cases are omitted because they were unsuitable. Great falls in the number of parasites occurred, even

without quinine, in Cases 5, 17 and 26; while, on the other hand small numbers of asexual forms remained in some cases for five or more days in spite of considerable daily dosage. In Case 23 a severe relapse began six days after the stoppage of quinine tannate which had been given for nine previous days in ten to thirty grain doses daily. No other relapses occurred after continuous quinine treatment; but twelve occurred without it.

10. *Observations on the sexual forms.* It is, of course, generally held—and probably quite rightly—that these are developed (by a cytological process not yet clearly seen) from the asexual forms. But at the same time no one has been able to note any correspondence between their numbers—many of the former may be present when the latter are very scarce, and vice versa. This rule, which is fully confirmed by our figures, has always been difficult to reconcile with the accepted theory of origin. On comparing our curves, however, the remarkable fact was observed (by D. T.) that there often seems to be indeed a correspondence, but that it is delayed for from eight to ten days—that is, that the sexual curve tends to rise that number of days after a rise in the asexual curve. Out of our twenty-five infections with *P. falciparum* eighteen showed crescents, and of these cases, 14, 22, 17, 18, 20, 23, 24 and 30 suggest this phenomenon, while in 17 and 20 the rises are sufficiently isolated to show it most distinctly. In Case 17, asexual rises existed on the 1st-3rd days, the 11th-12th and the 21st-22nd days, separated by apyrexial periods during which few asexual and no sexual forms were found; but on the 30th day (eight days after the last relapse) crescents appeared for the first time. In Case 20 an asexual relapse commenced on the 17th day and reached its height on the 20th day. A very few crescents had been found all the time; but a marked rise in their numbers commenced on the 26th day and reached its height on the 28th day. The most ready explanation is that the *crescents require eight to ten days for development*, during which they remain undetected, probably in the internal organs of the host. This is confirmed by the fact that numerous small ones are seen at the commencement of a rise; and also by the observation (frequently made) that in fresh infections the crescents seldom appear until a

week or more after the first attack of fever. Strong confirmation is also given by Case 6, in which Dr. Fantham noted a sudden appearance of sexual forms (*P. vivax*) on the 11th day, seven to ten days after the original asexual rise and after the patient had been taking thirty grains of quinine daily for a week—suggesting that the same phenomenon holds for *P. vivax* also.

Other observations are as follows:—(1) By no means every asexual rise is followed by a sexual one. In seven cases (13, 19, 25, 26, 28, 31, 33) no crescents at all were seen. In Case 13, a negro, 173,400 asexual forms per c.mm. failed to produce any; and none was observed in another negro (Case 28). (2) The number of crescents never exceeded five per cent. of asexual forms found in the generation which (hypothetically) produced them, and were generally in much smaller proportion. (3) We could find no relation to youth, vigour, amount of haemoglobin, duration of infection (from three to thirty weeks), or season of first infection. (4) The daily counts of crescents generally show marked daily variations, but we think that after considerable doses of quinine have been given for some days these daily variations tend to be smoothed out (Cases 16, 18, 22, 24). (5) In Cases 15, 14 and 16, particularly, a distinct tertian tendency was observed in the daily variations of crescents. This appears not to have been previously noticed, but is just what would be expected in the case of a tertian parasite—though, of course, the curve would be confused in a double tertian. (6) Sometimes the crescent curve suddenly rises with great rapidity, reaches its maximum, and then begins to fall on the next day with equal rapidity, though, later, the fall often tends to be much more slow (Cases 14, 15, 22, 23), especially when the numbers are very small. Apparently, the greater the maximum the quicker fall (Cases 14, 15); and the fall is sometimes jagged with a tertian tendency (ibid.). (7) Sometimes the maximum is irregularly maintained for a number of days (Cases 16, 18, 24), especially when quinine has not been previously given. (8) The crescents remained detectable for as many as 31, 32, 32, 35 and 44 days in Cases 20, 22, 23, 24 and 18 respectively; and finally disappeared (in 1 c.mm. of blood) in Cases 1, 11, 12, 16, 21 and 23.

From these data we think (1) that a varying percentage of asexual forms (sometimes very few or none) are constantly generating crescents, which, after about eight to ten days, appear in the peripheral blood. Hence, when the asexual forms remain sufficiently numerous for a period, as when no quinine is given, the stock of crescents is being constantly replenished. Thus the mortality among the older crescents is constantly being compensated for by new arrivals, and the total numbers appear to remain constant—an appearance which has given rise to the generally accepted hypothesis (perhaps erroneous) that the crescents survive for weeks. On the other hand the sudden rise and fall of their numbers seen in Cases 14, 15, 22 and 23, suggest that they really survive only for a short time after their appearance in the peripheral blood. In Cases 16 and 24, however, a few crescents were still found after quinine had been given continuously for sixteen to eighteen days, suggesting that some of them may be able to survive for longer periods. In Cases 20, 22 and 23, they appeared in increasing numbers for four to six days after continuous 30-grain doses of quinine had been commenced, suggesting that the drug has no effect upon them when they have once been generated. But quinine appears to affect the numbers of crescents indirectly by cutting off the source of supply. Though many researches upon the effect of quinine on crescents (a subject of the greatest sanitary importance) have been attempted, so far as we know the possibility of a constantly continued supply has not previously been taken into consideration. We are continuing researches on the point.

11. *The leucocytes* were counted frequently, but not always daily; at first by the haemocytometer, but in the later twenty cases by the thick film method. The latter method has the advantages of putting the leucocytes closer together and of enabling us to estimate them at the same time with the parasites, but, like the haemocytometer, it is not very appropriate for differential counts because of occasional uncertainty in distinguishing the various kinds. Accordingly these were made (in six cases) by thin films, 500 cells being examined on each occasion.

Our results are, as shown in all the cases, that during the pyrexial periods the total leucocytes are comparatively few, being frequently as low as 2 to 3,000 per c.mm. (Cases 17, 18, 23, 24, etc.). But as the asexual forms and the fever diminish the total leucocyte curve rises, and about seven days later exceeds the normal and may reach 20,000 and even, rarely, 30,000 per c.mm. (Case 7)—though this height is not maintained. The very high counts seem to be associated with much quinine. We observed generally that the so-called polymorphonuclear leucocytes, though rather few during the pyrexial periods, do not vary much from day to day, but about seven days after the fever has abated their numbers are markedly increased and vary much. The so-called mononuclear percentage was, however, always in excess throughout the cases, even long after disappearance of all forms of the parasites—for six weeks afterwards in Case 1, though quinine had been given daily during that period (out of hospital). We think, therefore, that a high mononuclear percentage is likely to be always of value in diagnosis in the absence of parasites. The normal ratio of all mononuclears to polymorphonuclears is about 35 per cent., but in our cases it was often 60 per cent., and in Case 30 reached 80 per cent. It tends to fall slightly with great improvement in health. Thus the total leucocyte rise appears to be due chiefly to mononuclears. During the individual daily paroxysms, as shown by Stephens and Christophers,* the total mononuclears are rather low during the height of the fever, but show a marked increase during the following remission, this increase affecting chiefly the large mononuclears. This process is repeated with each paroxysm, and if a paroxysm fails the mononuclears remain increased (Case 9 especially). But we could not always find these variations in the absence of fever and many parasites. This mononuclear reaction, which we have also seen in our case of trypanosomiasis, appears to be exactly comparable to the polymorphonuclear reaction described by F. W. Andrews ('Lancet,' June, July, 1910) as generally occurring in bacterial diseases.

12. *Various therapeutic agents.* Methylene blue was tried in three cases in 12 grain daily doses; the parasites diminished, but we cannot draw any conclusions from the figures. In Case 24, altogether 36 grains of soamin were injected intramuscularly in

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* The increase in the number of large mononuclear leucocytes as a diagnostic sign of malaria. Royal Society's Malaria Committee Reports, Fifth Series, 1901. Harrison & Sons, London.

5 to 10 grain doses on five occasions during twelve days, and yet a smart relapse occurred during and after the last dose. In the same case just before the same relapse X-rays had been applied over the spleen and abdomen for twenty minutes. The asexual parasite rise had commenced before the application. In Case 24, faradic and galvanic currents were applied over the spleen, each for ten minutes, but crescent counts made immediately before and two hours after the application remained unaltered

13. *Haemoglobin.* This was estimated in most of the later cases. The percentage always fell during fever and began to rise rapidly shortly afterwards, proving the destructive effect of the parasites and the rapid recuperation of the patients. The greatest fall was 25 per cent. after four days' fever (Case 20); but more detailed work is required to trace correspondence with the number of parasites. The lowest percentage found was 53 per cent. All the patients were anaemic at first, but the haemoglobin rose rapidly with improvement of health, and very rapidly in the more vigorous subjects. We decided not to attempt counts of red corpuscles, as these have been made so frequently already. The elimination of the haemoglobin and the findings in a case of blackwater fever will be dealt with in the two following papers by Dr. Simpson and in one by ourselves.

14. *Summary.* (1) There would seem to be a very decided correlation between the number of asexual *Plasmodia* found in the peripheral blood and the fever.

(2) As a rule, no fever exists unless the asexual forms exceed some hundreds per c.mm.

(3) The asexual forms do not always disappear between relapses (as often thought) but tend to persist in small numbers per c.mm., and often increase again for some days before the actual febrile relapse occurs.

(4) These observations give a coherent theory of the malarial invasion, according to which the infection is kept alive indefinitely by the ordinary sporulation of the asexual forms, and not by parthenogenesis or by resistant forms; and fever recurs only when the parasites are numerous enough to produce it.

(5) We estimate from our cases that considerable continued doses of quinine reduced the asexual forms by 50 to 80 per cent.

(6) There are strong reasons for supposing that the sexual forms require eight to ten days for development; that the often noticed long persistence of crescents is not due to their long life (as generally thought) but to constant replenishments of the stock by fresh broods; that they sometimes show a distinct tertian periodicity; and that quinine does not affect them when once generated; but ultimately reduces their numbers by destroying the generating cells. The sexual forms were never seen to produce fever.

(7) The leucocytes are below normal during febrile periods and above normal afterwards. The percentage of mononuclears rises after paroxysms and is always in excess of the normal.

(8) Methylene blue, soamin, X-rays, and faradic and galvanic currents had no results in a few experiments.

(9) The haemoglobin falls markedly with fever, but rises rapidly with convalesence.

(10) The faecal urobilin shows marked correlation with the occurrence of fever, and is specially studied in the accompanying paper by Dr. G. C. E. Simpson.

Many other deductions may be based on our observations, but will be better considered after more detailed counts have been made.

TABLE GIVING THE DAILY DETAILS OF 33 CASES OF MALARIA STUDIED BY ENUMERATIVE METHODS

Note.—The headline of each case gives initials, calling, age, where infected (presumably), how long previously infected (presumably), month of admission, and body weight on admission. The maximum temperatures are given in the Haematothermic Fahrenheit scale (H.F.), which equals the excess over 95°F., multiplied by 10. The leucocytes are recorded in hundreds per c.mm.; the haemoglobin either in the Talqvist or the Sahli scale, the urobilin (either urinary or faecal) in milligrams; the drugs in grains; and the parasites (either all forms together or asexual and sexual forms separately) in numbers per c.mm.

A. *P. malariae* and *P. falciparum* (crescents only).

Case 1.—W. M., ship butcher, 42; West Africa; 70 days; February, 145 lbs.

Days	1	2	3	4	5	6	[illegible]
Total parasites	—	254	76	—	148	121	[illegible]
Crescents	200	164	170	—	180	141	[illegible]
Maximum temp.	26	20 (rigor)	20	22	30	34 (rigor)	[illegible]
Methylene blue, grs.	—	—	—	—	4	12	12
Polychr. methylene blue, c.c. ...	—	—	—	—	—	—	—
Ur. urob., mgr.	20	?	18	0	0	—	—
Days	8	9	10	11	12	13	[illegible]
Total parasites	127	140	57	17	17	23	[illegible]
Crescents	220	117	86	70	51	38	[illegible]
Maximum temp.	40	30	30	30	16	22	[illegible]
Methylene blue, grs.	12	12	12	12	12	12	[illegible]
Polychr. methylene blue, c.c. ...	–	—	—	—	—	—	—
Ur. urob., mgr.	—	—	—	—	—	—	—
Days	15	16	17	18	19	20	21
Total parasites	16	6	0	—	0	0	[illegible]
Crescents	10	10	0	-	2	2	2
Maximum temp.	34	30	34	24	30	30	22
Methylene blue, grs.	—	—	—	—	4	12	12
Polychr. methylene blue, c.c. ...	15	45	30	0	—	—	—
Ur. urob., mgr.	—	—	—	—	—	-	—

B. *P. vivax.*

Case 2.—J. B., seaman, 26; Colon; 120 days; January, 147 lbs.

Days	1	2	3	4	5	6	7
Total parasites	8,766	6,233	2,630	1,500	1,260	—	0
Maximum temp.	48	38	58	20	24	24	18
Q. hydrochloride, grs.	0	15	30	30	30	30	30

Case 3.—J. H., seaman, 50; W. Africa; 35 days; January, ?

Days	1	2	3	4	5	6	to 11
Total parasites	11,019	1,444	642	88	40	0	0
Maximum temp.	84	42	44	34	32	22	normal
Q. sulph., grs.	—				20	20	20
Ur. urob., mgs.	2	0	0	0	0	—	—

Case 4.—J. Hough, ship steward, 22; Vera Cruz; 112 days; March, 105 lbs.

Days	1	2	3	4	5	6	7
Total parasites	370	308	505	280	46	8	0
Maximum temp.	46	44	76	58	30	26	28
Q. hydrochloride, grs.	—	—	-	30	30	30	30
Total leucocytes	—	55	—	86	—	—	—
Hb. Tal., percentage	90	85	85	80	85	85	85
Ur. urob., mgs.	54	0	18	82	140	17	19
Days	8	9	11	-	—	—	—
Total parasites	0	0	0	—	—	—	—
Maximum temp.	20	24	14		—	—	—
Q. hydrochloride, grs.	30	30	30	—	-	—	-
Total leucocytes	84	—	160	—	—	—	—
Hb. Tal., percentage	85	85	90				—
Ur. urob., mgs.	0	0	—	—	—	—	—

Case 5.—R. R., planter, 25; Fernando Po; 75 days, March, 129 lbs

Days	1	2	3	4	5	6	7
Total parasites	28,700	7,753	1,153	1,000	150	200	140
Maximum temp.	92	60	76	50	30	36	26
Methylene blue, grs.	—		-	12	12	12	12
Total leucocytes	—	65	50	56	71	47	48
Hb. Tal., percentage	—	90	85	80	85	85	80
Ur. urob., mgs.	19	14	23	12	—	-	-
Faec. urob., mgs.	0	27	0	0	-		—

Case 5—continued

Days	8	9	10	11	12	13	14
Total parasites	200	64	96	32	78	[illegible]	[illegible]
Maximum temp.	20	28	32	24	18	16	[illegible]
Methylene blue, grs.	12	12	12	12	—	—	—
Total leucocytes	—	43	—	—	—	—	—
Hb. Tal., percentage	90	90	90	90	90	90	90
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—		—	—	

Days	15	16					
Total parasites	6	30		—	—	—	—
Maximum temp.	28	22	—	—	—	—	—
Methylene blue, grs.	—	—	—	—	—	—	—
Total leucocytes	78	—	—	—	—	—	—
Hb. Tal., percentage	90	90	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 6.—J. M., sailor, 19, Niger, 135 days; April, ?

Days	1	2	3	4	5	6	[illegible]
As. parasites	852	668	1,080	256	64	68	44
Sex. parasites	—	—	—	—	—	—	—
Maximum temp. . ..	26	70	102	82	20	24	20
Q. bihydrochloride, grs. ...	0	0	0	30	30	30	30
Ur. urob., mgs.	—	18	1	115	15	0	15
Faec. urob., mgs.		—	—	286	403	130	[illegible]

Days	8	9	10	11	12	13	14
As. parasites	24	8	28	—	—	—	—
Sex. parasites	—	—	—	412	144	56	[illegible]
Maximum temp.	24	16	20	14	20	26	[illegible]
Q. bihydrochloride, grs. ...	30	30	30	30	30	30	30
Ur. urob., mgs.	0	0	—	—		—	
Faec. urob., mgs.	100	—	—		—	—	20

Case 6—continued

Days	15	16	17	18	--		
As. parasites	–		--			—	
Sex. parasites	48	52	20	8			
Maximum temp.	24	10	24	34			
Q. bihydrochloride, grs. ..	30	30	30	30	–		
Ur. urob., mgs.			--	—	–		—
Faec. urob., mgs.	—	-	—	-		—	—

Case 7.—W. M., fireman, 35; Niger; 90 days; May, 155 lbs.

Days	1	2	3	4	5	6	7
Total parasites	-	440	1,300	133	250	152	148
Maximum temp.	22	102	66	34	30	28	26
Q. hydrochloride, grs.	0	10	0	0	0	0	0
Total leucocytes	20	40	22	85	110	56	150
Hb. Tal., percentage	70	80	75	85	90	85	80
Faec. urob., mgs.	—	0	530	340	88	0	200

Days	8	9	10	11	12	13	14
Total parasites	28	96	100	130	80	124	228
Maximum temp.	26	24	34	24	26	30	28
Q. hydrochloride, grs.	0	0	0	0	0	0	0
Total leucocytes	215	151	59	62	230	149	110
Hb. Tal., percentage	85	85	—	80	90	90	—
Faec. urob., mgs.	130	0	0	0	70	50	-

Days	15	16	17	18	19	20	21
Total parasites	284	368	540	280	400	440	50
Maximum temp.	42	54	22	30	20	32	20
Q. hydrochloride, grs.	0	10	30	30	30	30	30
Total leucocytes	61	100	70	80	24	71	45
Hb. Tal., percentage	80	80	80	85	Sahli per cent.	80	80
Faec. urob., mgs. ...							

Case 7—continued

Days	22	23	24	25	26	27	[illegible]
Total parasites	16	28	32	0	12	0	[illegible]
Maximum temp. ..	32	22	28	28	32	34	[illegible]
Q. hydrochloride, grs. . .	30	30	30	30	30	30	
Total leucocytes .. .	80	180	44	45	50	140	[illegible]
Hb. Tal., percentage .	83	83	82	80	75	80	[illegible]
Faec. urob., mgs. ..	—	—	—	—	—	—	—

Days	29	30	31	32	33	34	[illegible]
Total parasites	0	[illegible]	0	0	0	[illegible]	
Maximum temp.	30	36	30	38(?)	28	28	[illegible]
Q. hydrochloride, grs.	30	30	30	30	30	30	[illegible]
Total leucocytes .. .	100	61	200	75	160	300	-
Hb. Tal., percentage	75	80	75	75	80	83	—
Faec. urob., mgs.	—	—	—	—	-	—	-

Days	36	37	38	—	—	-	-
Total parasites	0	0	0	—	—	—	-
Maximum temp.	30	52	—		—	—	—
Q. hydrochloride, grs.	30	30	30	—	—	-	-
Total leucocytes	320	185	160	—	.	-	—
Hb. Tal., percentage	95	100	100	—	-	—	—
Faec. urob., mgs.	—	-	—	-	—	-	-

Case 8.—P. D., fireman, 29; ?, 240 days; May, ?

Days	1	2	3	4	5	6	-
Total parasites	7,600	3,615	2,816	168	50	30	[illegible]
Maximum temp.	88	86	90	26	24	16	[illegible]
Q. hydrochloride, grs.	—	—	10	30	*et. seq.*	—	—
Total leucocytes	78	30	28	40	45	27	[illegible]
Hb. Sah., percentage	80	68	80	80	75	80	[illegible]
Ur. urob., mgs.	0	80	111	80	—	-	—
Faec. urob., mgs.	0	0	1,120		-	-	—

Case 8—*continued*

Days	8	9	10	11	12	13	14
Total parasites	16	0	0	8	0	4	0
Maximum temp.	30	24	26	28	20	20	24
Q. hydrochloride, grs.	–		—				
Total leucocytes	56	115	300	400	240	200	125
Hb. Sah., percentage	70	78	75	75	77	70	80
Ur. urob., mgs.				—			—
Faec. urob., mgs.					—		—

Days	15	16	17	18	19	20	21
Total parasites	0	0	0	0	0	0	0
Maximum temp.	34	30	24	22	30	34	
Q. hydrochloride, grs.						–	
Total leucocytes	150	8	110		270	96	250
Hb. Sah., percentage	85	8–	–		85	95	100
Ur. urob., mgs.						—	—
Faec. urob., mgs.						–	

Case 9.—N. B., sailor, 24 ; Peru, 45 days ; June, 161 lbs.

Days	1	2	3	4	5	6	7
Total parasites	15,400	9,600	12,180	9,600	50	40	0
Maximum temp.	103	32	97	34	36	22	25
Q. hydrochloride, grs.		—	20	30	*et seq*	—	—
Mon. leucocytes	15	75	8	72	116	36	65
Pol. leucocytes	41	40	3	42	25	40	65
Hb. Sah., percentage	95	90	90	95	90	85	85

Days	8	9	10	11	12	—	
Total parasites	20	0	0	0	0	—	–
Maximum temp.	22	30	25	25	–		
Q. hydrochloride, grs.					—		
Mon. leucocytes	35	45	48	105	48		
Pol. leucocytes	35	32	75	132	35		
Hb. Sah., percentage	80	90	90	85	90		—

C. *P. falciparum*—

Case 10.—A. M. ship engineer, 37; Kamerun, 140 days; January, 145 lbs.

Days	1	2	3	4	5	6
*As. parasites	1,000	10,000	14,700	8,650	2,600	0
Crescents			—	—		137
Maximum temp.	62	58	38	54	26	20
Q. sulph., grs.				20	20	20
Ur. urob., mgs.		100	75	75	155	45
Days	8					
As. parasites						—
Crescents	13					
Maximum temp.	18					
Q. sulph., grs.	20		—		—	—
Ur. urob., mgs.	17				—	—

Case 11.—W. M., steward, 17; Niger; 190 days; January, 126 lbs.

Days	1	2	3	4	5	6
As. parasites	56,257	55,300	0	0	0	0
Crescents			4	16	0	15
Maximum temp.	90	68	44	18	20	16
Q. hydrochloride, grs.	30	30	30	30	30	30
Ur. urob., mgs.	75	1,730	500	250	237	120
Days	8	9	10	11	12	13
As. parasites			—	0	0	
Crescents	(*et seq.* 5 days)					—
Maximum temp.	30	24	34	24	20	24
Q. hydrochloride, grs.	15	15	15	15	15	15
Ur. urob., mgs.	55	0	0	50	20	

Case 12.—C. H., seaman, 24; Niger, 30 days; January, 112 lbs.

Days	1	2	3	4	5	6
As. parasites	24,600	47,000	45,250	2,250	0	0
Crescents		—	—	—	180	168
Maximum temp.	50	86	64	42	32	20
Q. sulph., grs.	—	—	20	20	20	20
Ur. urob., mgs.	128	133	125	143	51	50

* As. = Asexual.

Case 12—*continued*

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	0	0	0	0
Crescents	48	72	25	26	34	37	9
Maximum temp.	20	18	20	18	20	18	12
Q. sulph., grs.	10	10	10	10	10	10	10
Ur. urob., mgs.	0	0	40	18	0	27	17

Days	15	16	17	—	—	—	—
As. parasites	—		—	—	—		—
Crescents	8		5	—	—	—	—
Maximum temp.	22	12	12	—	—	—	—
Q. sulph., grs.	—	10	10	—	—	—	—
Ur. urob., mgs.	0	0	—	—	—	—	—

Case 13.—G. N., negro seaman, 22; Congo; 30 days; January, 118 lbs.

Days	1	2	3	4	5	6	7
As. parasites	181,933	173,400	60,100	673	0	0	0
Crescents	0	0	0	0	0	0	0
Maximum temp.	108	100	46	50	26	20	20
Q. hydrochloride	30	30	30	30	30	30	30
Ur. urob., mgs.	—		680	100	100	100	60

Days	8	9	—	—	—	—	—
As. parasites	0	0	—	—	—	—	—
Crescents	0	0	—	—	—	—	—
Maximum temp. .. .	20	20	—	—	—	—	—
Q. hydrochloride .	15	—	—	—	—	—	—
Ur. urob., mgs.	20	112	0 *et. seq.* (14 days)				

Case 14.—J. M., sailor, 31; Gold Coast; 56 days; January, 158 lbs.

Days	1	2	3	4	5	6	7
As. parasites	28,100	30,050	410	0	0	0	100
Crescents	—		—	565	1,190	2,000	1,200
Maximum temp.	76	30	44	14	20	16	18
Q. bihydrochloride, grs. .	10	20	0	10	0	—	
Total leucocytes ...	78	87	81	56	62	75	50
Ur. urob., mgs.	0	4	0	20	24	27	30

Case 14—*continued*

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	0	0	0	
Crescents	1,500	1,300	1,090	1,500	1,108	1,130	[illegible]
Maximum temp.	20	14	14	12	18	10	—
Q. bihydrochloride, grs. ...				—	—	—	
Total leucocytes	56	81	80	56	59	62	[illegible]
Ur. urob., mgs.	39	11	9				

Days	15	16	17	18	19	[illegible]	21
As. parasites	0	0	0	0	0	0	0
Crescents	1,022	691	336	422		162	[illegible]
Maximum temp.	—	—	—			—	
Q. bihydrochloride, grs. ...	—	—	—	—	—	—	
Total leucocytes	50	68	59	—	81	56	—
Ur. urob., mgs.	—	—	—	—	—		—

Case 15.—W. B., fireman, 27; Vera Cruz; 49 days; January, 126 lbs.

Days	1	2	3	4	5	6	[illegible]
As. parasites	0	—	—		—		
Crescents	6,700	—	4,370	4,700	4,000	—	2,200
Maximum temp.	16	14	22	normal	—	—	
Q. hydrochloride, grs.	30	30	30	30	15	*et seq.*	—
Total leucocytes	112	—	100	87	97	—	125
Ur. urob., mgs.	0	0	—	—	—		—

Days	8	9	10	11	12	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	1,870	937	937	770	470	—	—
Maximum temp.	—	—	—	—	—	—	—
Q. hydrochloride, grs.	—	—	—	—		—	—
Total leucocytes	168	137	134	121	147		—
Ur. urob., mgs.	—	0	0	—	—	—	—

Case 16.—J. G., seaman, 17; W. Africa; 70 days; February, 126 lbs.

Days	1	2	3	4	5	6	7
As. parasites	0		—	—		—	
Crescents	244	107	99	230	146	220	197
Maximum temp.	18	24	14	normal		—	—
Total leucocytes	75	81	93	112	122	137	115
Q. sulph., grs.		—			—	—	—

Days	8	9	10	11	12	13	14
As. parasites	—	—			—	—	—
Crescents	94	200	107	125	152	97	140
Maximum temp.			—			—	—
Total leucocytes	78	128	107	112	93	106	100
Q. sulph., grs.	—	—		20	20	10	10

Days	15	16	17	18	19	20	21
As. parasites	—	—	—	—	—	—	—
Crescents	86	60	47	65	44	51	32
Maximum temp.		—	—	—		—	—
Total leucocytes	122	100	93	131	134	137	—
Q. sulph., grs.	30	30	*et seq.*				

Days	22	23	24	25	26	—	
As. parasites		—	—	—		—	—
Crescents	8	10	20	10	6	—	—
Maximum temp.	—				—	—	—
Total leucocytes	—	—	—	—	—	—	—
Q. sulph., grs.	—			—	—		—

Case 17.—R. B., seaman, 19; Congo; 49 days; March, 134 lbs.

Days	1	2	3	4	5	6	7
As. parasites	5,250	380	2,837	336	288	184	60
Crescents	0	—	—	—	—	—	—
Maximum temp.	70	70	40	34	28	26	22
Q. hydrochloride	—		—	—	—	—	—
Total leucocytes	31	25	—	—	25	—	—
Hb. Tal., percentage	70	60	65	70	75	75	75
Ur. urob., mgs.	33	69	90	51	190	43	43
Faec. urob., mgs.	—		0	1,140	—	—	—

Case 17—continued

Days	8	9	10	11	12	13	14
As. parasites	36	55	12	1,750	360	0	[illegible]
Crescents		-	-	—	—	—	
Maximum temp.	20	22	32	64	44	30	[illegible]
Q. hydrochloride	-	—	—	20	—	—	-
Total leucocytes		-	—	26	—	-	
Hb. Tal., percentage	75	80	80	75	70	75	[illegible]
Ur. urob., mgs.	64	75	70	29	183	25	[illegible]
Faec. urob., mgs.	—	—	-	—	—	453	—

Days	15	16	17	18	19	20	21
As. parasites	0	12	0	0	28	60	1,32
Crescents	—	—	—	—	—	—	-
Maximum temp.	18	20	20	18	22	27	[illegible]
Q. hydrochloride	—		—	—	-	-	10
Total leucocytes	68	—	—	—	68	-	-
Hb. Tal., percentage	80	80	80	85	85	85	[illegible]
Ur. urob., mgs.	76	68	76	19	25	72	[illegible]
Faec. urob., mgs.	—	-	—	—	-	—	[illegible]

Days	22	23	24	25	26	27	28
As. parasites	1,250	0	8	0	0	0	[illegible]
Crescents	—	—	—	—	—	—	—
Maximum temp.	50	30	20	22	18	16	22
Q. hydrochloride	10	—		-	—	10	[illegible]
Total leucocytes	40	—	—	—	—	—	—
Hb. Tal., percentage	80	75	80	-	85	-	90
Ur. urob., mgs.	103	68	44	42	70	70	—
Faec. urob., mgs.	-	-	—	—	—	—	—

Case 17—*continued*

Days	29	30	31	32	33	34	35
As. parasites	0	0	—	—	—	—	—
Crescents	—	25		0	—	12	—
Maximum temp.	18	22	16	18	18	28	22
Q. hydrochloride .. .	30	*et seq.*		—		—	—
Total leucocytes	125	—			—	—	—
Hb. Tal., percentage ...		90		85	—	90	
Ur. urob., mgs.		45	0		—	—	—
Faec. urob., mgs.			—			—	—
Days	36	37	38	39	40		
As. parasites	—		—	—	—	—	
Crescents	0	—	—	12	—	—	—
Maximum temp.	26	30	28	26	24	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Total leucocytes		—	—	—	—	—	—
Hb. Tal., percentage	90		90	—	90	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 18.—A. C., steward, 29, Congo; 77 days: March, 100 lbs.

Days	1	2	3	4	5	6	7
As. parasites	32	—	0	—	24		128
Crescents	32	—	15		24	—	36
Maximum temp. .. .	32	32	30	32	26	20	26
Q. hydrochloride			—	—	—	—	—
Ur. urob., mgs.	—	—	116	47	81	54	38
Days	8	9	10	11	12	13	14
As. parasites	—	26	—	80	64	—	388
Crescents	—	28	—	68	52	—	52
Maximum temp. ...	20	28	28	24	20	10	10
Q. hydrochloride	—		—	—	—	—	—
Ur. urob., mgs.	33	7	15	10	—	—	—

Case 18—continued

Days	15	16	17	18	19	20	21
As. parasites	—	1,860	20	744	60	0	—
Crescents		72	32	16	24	20	34
Maximum temp.	20	38	38	26	16	20	[illegible]
Q. hydrochloride ...	—	—	5	5	5	5	5
Ur. urob., mgs.	—	—	—	—	30	32	12

Days	22	23	24	25	26	27	28
As. parasites	16	0	0	0	0	0	0
Crescents	92	180	128	196	228	264	212
Maximum temp.	18	26	22	26	16	26	24
Q. hydrochloride	5	5	5	5	5	5	5
Ur. urob., mgs.	10	0	0	—	0	—	20

Days	29	30	31	32	33	34	35
As. parasites	0	30	32	0	0	—	—
Crescents	276	286	138	208	136	128	100
Maximum temp.	24	84	30	22	30	32	30
Q. hydrochloride	5	10	30	30	*et seq.*	—	—
Ur. urob., mgs.	0	0	—	—	—	—	—

Days	36	37	38	39	40	41	42
As. parasites	—	—		—	—	—	—
Crescents	50	50	—	48	—	16	—
Maximum temp.	20	42	22	24		—	—
Q. hydrochloride	—	—	—	—	—	—	—
Ur. urob., mgs.	—	—	—		—	—	—

Days	43	—	—	—	—	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	8	—	—	—	—	—	—
Maximum temp.	—	—	—	—	—	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 19.—H. R., sailor, 20; Niger; 40 days; March, 110 lbs.

Days	1	2	3	4	5	6	7
As. parasites	3,310	2,543	184	0	4	8	0
Crescents	None			—		—	
Maximum temp.	76	75	32	32	22	16	20
Q. hydrochloride		20	—		—		—
Total leucocytes	37	31	—		—	51	—
Hb. Tal., percentage	60	50	65	70	70	75	80
Ur. urob., mgs.	67	0	132	144	170	115	39

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	0	24	0	140
Crescents	—	—	—				—
Maximum temp.	28	24	24	32	24	42	32
Q. hydrochloride	—	—		—	—	—	—
Total leucocytes		—		94	—	—	—
Hb. Tal., percentage	8	80	85	85	80	80	85
Ur. urob., mgs.	108	116	38	40	42	0	38

Days	15	16	17	18	19	20	21
As. parasites	200	—	0		0	—	0
Crescents			—	—	—		—
Maximum temp.	54	36	26	20	20	22	18
Q. hydrochloride	10	30	30	*et seq.*			—
Total leucocytes	—		128	—		—	—
Hb. Tal., percentage	80	70	80		80		80
Ur. urob., mgs.	—	116	30	13	0	—	—

Case 20.—F. B., steward, 24; Niger; 40 days, March, 129 lbs.

Days	1	2	3	4	5	6	7
As. parasites	4,172	580	22	20	4	0	0
Crescents	0	0	0	0	0	4	12
Maximum temp.	90	56	70	40	24	22	20
Q. hydrochloride	10	0	20		—		—
Total leucocytes	—			37	—		60
Hb. Tal., percentage	60	55	60	75	75	80	75
Ur. urob., mgs.	0	273	182	162	81	54	29

Case 20—continued

Days	8	9	10	11	12	13	[illegible]
As. parasites	0	[illegible]	12	0	0		
Crescents	8	16	12		0		—
Maximum temp.	26	28	24	24	26	20	[illegible]
Q. hydrochloride				—		—	—
Total leucocytes		[illegible]	100	-			
Hb. Tal., percentage	80	80	80		85	80	—
Ur. urob., mgs.	32	40	26	0	0	70	-
Days	15	16	17	18	19	20	21
As. parasites	32		200		2,698	4,000	3,750
Crescents	0		4	-	0	0	[illegible]
Maximum temp.	22	22	26	40	74	70	[illegible]
Q. hydrochloride				-	—	10	30
Total leucocytes	150	-		—	90	46	—
Hb. Tal., percentage	85	—	80	—	83	83	S. per cent.
Ur. urob., mgs.	—	0		8	72	107	81
Days	22	23	24	25	26	27	28
As. parasites	1,560	104	0	—	0	—	320
Crescents	0	0	0	—	32		88
Maximum temp.	38	32	24	24	26	24	16
Q. hydrochloride	30	30	30	30	30	30	30
Total leucocytes	42	35	35	—	—	—	—
Hb. Tal., percentage	55	60	70	—	80	—	80
Ur. urob., mgs.	29	7	10	23	21	0	8
Days	29	30	31	32	33	34	35
As. parasites		0		0		0	—
Crescents	—	68	—	56	—	8	-
Maximum temp.	20	24	18	24	24	20	20
Q. hydrochloride	30	30	30	30	20	20	20
Total leucocytes	—	170	—	300		210	—
Hb. Tal., percentage	-	80	—	85	—	90	-
Ur. urob., mgs.	[illegible]	[illegible]					

Case 20—*continued*

Days	36	37	—	—	—	—	—
As. parasites	—	0	—		—		—
Crescents		36		—	—	—	
Maximum temp.	20	24	—	—	—		—
Q. hydrochloride	20	20					—
Total leucocytes	—	264	—	—			—
Hb. Tal., percentage		90			—		
Ur. urob., mgs.	—						—

Case 21.—W. T., seaman, 50; Niger . 28 days; April, 158 lbs.

Days	1	2	3	4	5	6	7
As. parasites	36,000	25,300	16,000	280	—	0	300
Crescents	0	0	0	28	28	24	24
Maximum temp.	61	58	52	32	20	20	24
Q. bihydrochloride	—	—	20	30	30	30	—
Total leucocytes	—	25	—	43	—	—	—
Ur. urob. mgs.	—	10	48	1,300	273	54	40
Faec. urob., mgs.			0	0	30	4,100	?
Days	8	9	10	11	12	13	14
As. parasites	—	0	—	0	—	0	—
Crescents	—	16	—	4	—	8	—
Maximum temp.	24	34	26	26	22	26	24
Q. bihydrochloride	—		—		16	20	20
Total leucocytes	—	55	—	34	—	54	—
Ur. urob., mgs.	39	19	—		10	—	—
Faec. urob., mgs.	0	333	—		—	—	—
Days	15	16	17	18	19	20	—
As. parasites			0		—	0	—
Crescents	—	—	4	—	—	0	—
Maximum temp.	16	30	24	28	22	24	—
Q. bihydrochloride	20	20	10	10	10	10	—
Total leucocytes			75			119	
Ur. urob., mgs.	—				—		—
Faec. urob., mgs.	—		—	—	153	—	—

Case 22.—C. G., sailor, 30; Niger; 40 days; April.

Days	1	2	3	4	5	6	[illegible]
As. parasites	7,920		20,250	44,800	920	30	[illegible]
Crescents	24		32	56	224	584	[illegible]
Maximum temp. . . .	64	82	76	70	36	18	[illegible]
Q. bihydrochloride		10	30	30	30	30	[illegible]
Total leucocytes	—	—	—	130	200	80	[illegible]
Hb. Tal., percentage		85	80	82	80	80	[illegible]
Ur. urob., mgs.	0	0	1,500	0	50	70	[illegible]
Faec. urob., mgs.	1,730	780	0	1,670	0	?	[illegible]

Days	8	9	10	11	12	13	[illegible]
As. parasites	0	0	0	?	?	0	[illegible]
Crescents	1,128	1,180	1,384	1,288	1,240	948	[illegible]
Maximum temp.	24	24	18	26	14	24	18
Q. bihydrochloride	30	30	30	30	30	30	[illegible]
Total leucocytes	80	50	70	75	60	60	140
Hb. Tal., percentage		75	77	75	73	65	[illegible]
Ur. urob., mgs.	45	95	100	120	14	9	[illegible]
Faec. urob., mgs.	?	0	0	0	120	0	[illegible]

Days	15	16	17	18	19	20	[illegible]
As. parasites	0	0	0	0	0	—	—
Crescents	856	824	612	336	508	264	132
Maximum temp.	10	16	26	24	30	30	[illegible]
Q. bihydrochloride	30	30	30	10	10	20	—
Total leucocytes	100	78	60	180	90	140	[illegible]
Hb. Tal., percentage	75	80	85	—	85	—	85
Ur. urob., mgs.	23	38	18	0	12	0	[illegible]
Faec. urob., mgs.	220	386	113	0	?	0	—

Case 22—continued

Days	22	23	24	25	26	27	28
As. parasites	—		0	—	—	—	—
Crescents	190	120	100	40	32	-	20
Maximum temp.	10	16	14	22	20	10	10
Q. bihydrochloride	-	-	—	-	-	-	10
Total leucocytes	150	115	135	106	70	-	78
Hb. Tal., percentage	-	80		80	80	75	78
Ur. urob., mgs.	-			33	27	52	38
Faec. urob., mgs.	—	—	- -	0	0	720	373

Days	29	30	31	32	33	34	
As. parasites			-	-		-	
Crescents	—	4	-	0	—	-	
Maximum temp.	20	10	14	10	—		
Q. bihydrochloride	10	10	10	10		—	-
Total leucocytes	—	100	—	160	—		—
Hb. Tal., percentage	84	85	82	82	-	—	—
Ur. urob., mgs.	10	0	—	-	-	—	- -
Faec. urob., mgs.	0	0	?	0	0	10	—

Case. 23—T. C., sailor, 17; Niger; 21 days; April, 110 lbs.

Days	1	2	3	4	5	6	7
As. parasites	24,000	5,520	1,700	500	1,200	160	—
Crescents	- -	-	- -	—	—	160	—
Maximum temp.	98	90	32	30	28	14	14
Q. bihydrochloride	20	—	—	—	—	—	
Total leucocytes	52	—		—	30	—	—
Hb. Tal., percentage	85	80	80	80	80	85	—

Days	8	9	10	11	12	13	14
As. parasites	28,500	22,600	1,600	340	0	0	0
Crescents	608	635	720	780	552	368	432
Maximum temp.	86	100	86	30	32	20	20
Q. bihydrochloride	tannate	10	20	30	30	30	30
Total leucocytes	-	48	—	—	40	52	—
Hb. Tal., percentage	75	75	75	73	65	70	—

Case 23—continued

Days	15	16	17	18	19	20	[illegible]
As. parasites	0	0	0	0		480	-
Crescents	500	464	468	468	-	344	
Maximum temp.	28	20	32	20	30	28	[illegible]
Q. bihydrochloride	30	30	20	—		—	
Total leucocytes		102		-		—	-
Hb. Tal., percentage	85	85	85	85		85	

Days	22	23	24	25	26	27	[illegible]
As. parasites	2	-	10,948	11,360	1,600	1,584	[illegible]
Crescents	160	-	152	20	24	[illegible]	[illegible]
Maximum temp.	28	30	86	90	54	22	[illegible]
Q. bihydrochloride		—		30	30	*et seq.*	-
Total leucocytes	—	—	62	60	34	33	[illegible]
Hb. Tal., percentage	80		80	80	75	80	[illegible]

Days	29	30	31	32	33	34	[illegible]
As. parasites	0	0	0	0	100	0	[illegible]
Crescents	8	4	20	24	16	4	[illegible]
Maximum temp.	20	20	32	32	20	24	[illegible]
Q. bihydrochloride	—	—	—	—	—	-	—
Total leucocytes	—	48	80	130	98	110	[illegible]
Hb. Tal., percentage	85	82	82	85	85	85	[illegible]

Days	36	37	—	—	-	-	-
As. parasites	—	0			—	-	
Crescents	—	0		—	-	—	-
Maximum temp.	30	20		-	—	—	-
Q. bihydrochloride		—			—	-	-
Total leucocytes	-	65	—	—	—	—	-
Hb. Tal., percentage	—	90	—	—	-	-	-

Case 24.—J. K., steward, 29; Niger; 28 days; April, 126 lbs.

Days	1	2	3	4	5	6	7
As. parasites	5,190	900	1,000	450	900	720	25,000
Crescents	0	0	20	16	88	168	540
Maximum temp. . ..	52	38	26	32	24	52	24
Q. hydrochloride	—	—	—			—	10
Soamine, grs.							
Total leucocytes		-	-	-	60	80	58
Hb. Tal., percentage . .	80	80	80	75	72	80	75
Ur. urob., mgs.	—	48	24	11	20	10	0

Days	8	9	10	11	12	13	14
As. parasites	1,000	9,500	3,900	600			—
Crescents	672	348	548	500	532	564	636
Maximum temp.	80	60	30	36	34	34	32
Q. hydrochloride . . .		-	10	-			-
Soamine, grs.	10		8	-	—	8	-
Total leucocytes	36	45	70	50	70	140	160
Hb. Tal., percentage	70	75	75	70	75	80	80
Ur. urob., mgs.	0	—	-		-	—	—

Days	15	16	17	18	19	20	21
As. parasites	0	0	200	2,800	1,600	8,300	15,000
Crescents	784	572	520	630	496	367	212
Maximum temp.	24	26	38	40	44	54	54
Q. hydrochloride	—	—	—	-		10	30
Soamine, grs.	—	—	5	—	5	—	—
Total leucocytes	75	138	70	45	70	68	60
Hb. Tal., percentage	80	82	82	82	75	75	80
Ur. urob., mgs.	(faecal)	140	—	—	(faecal)	80	—

Days	22	23	24	25	26	27	28
As. parasites	5,600	200	1,000	1,100	0	0	0
Crescents	200	188	132	108	92	128	68
Maximum temp.	54	32	20	34	32	30	24
Q. hydrochloride	30	*et seq.*		—	—	-	—
Soamine, grs.	0	0	—	—	—	—	—
Total leucocytes .. .	35	55	42	36	78	56	120
Hb. Tal., percentage	80	82	82	80	82	80	85
Ur. urob., mgs.		—	—	-		—	—

Case 24—continued

Days	29	30	31	32	33	34	
As. parasites	-	0		0	—	0	
Crescents	—	28	—	40	—	16	—
Maximum temp.	30	30	24	32	18	22	
Q. hydrochloride			–	—	-		—
Soamine, grs							
Total leucocytes	–	90		200		160	—
Hb. Tal., percentage		85		85		85	-
Ur. urob., mgs.		—	—	-	—	—	-

Days	36	37	38	39			—
As. parasites	0	–	0	0			
Crescents	12		8	0		-	—
Maximum temp.	30	26	22	26		—	-
Q. hydrochloride				–		—	-
Soamine, grs.				—		-	-
Total leucocytes	115	–	200	195	–	–	-
Hb. Tal., percentage	—	-	90	90	—	—	—
Ur. urob., mgs.		–	–		—	—	-

Case 25.—K. K., sailor, 20; Gambia; 20 days; April, 154 lbs.

Days	1	2	3	4	5	6	
As. parasites	3,000	4,000	3,956	14,076	13,392	18,000	2,10
Crescents	0	0	0	0	0	0	
Maximum temp.	86	88	70	98	74	74	
Q. bihydrochloride			-	10	20	30	30
Ur. urob., mgs.	76	30	10	—	–	–	-
Faec. urob., mgs.	120		—	-	—	—	

Days	8	9	10	11	12	13	14
As. parasites	2,304	684	2,200	1,120	672	400	
Crescents	0	0	0	0	0	0	
Maximum temp.	36	30	32	28	26	26	
Q. bihydrochloride	*et seq.*	–		—	—	-	—
Ur. urob., mgs.	—	–	-	—	-	—	—
Faec. urob., mgs.	—	–	—	-	—	—	—

Case 26.—A. M., sailor, 44; Niger; 20 days; May, 168 lbs.

Days	1	2	3	4	5	6	7
As. parasites	9,700	3,500	2,500	?	?	80	-
Crescents	0	*et seq.*		—	—	—	—
Maximum temp.	56	24	14	20	26	24	30
Q. hydrochloride	—	—			—	—	10
Total leucocytes	66	82	60	90	130	120	124
Hb. Tal., percentage	80	80	80	85	90	90	90
Ur. urob., mgs.	22	18	19	26	15	10	0

Days	8	9	10	11	12	13	14
As. parasites	400	2,000	300		0	0	0
Crescents	—			—	-	—	—
Maximum temp.	22	46	38	24	12	20	34
Q. hydrochloride	30	*et seq.*			—	—	-
Total leucocytes	160	155	176	168	150	120	—
Hb. Tal., percentage	90	80	88	88	84	90	—
Ur. urob., mgs.	10			—	—	—	—

Days	15	16	17	18	-	—	—
As. parasites	0	0		0	—	—	—
Crescents	-	—	—	—	—	—	—
Maximum temp.	16	10	20	24	-	—	—
Q. hydrochloride	...	—	—	—	—	—	—
Total leucocytes	200		178	—	—	—	—
Hb. Tal., percentage	90	—	90	—	—	—	—
Ur. urob., mgs.	—			—	—	—	—

Case 27.—S. P., trader, 30; W. Africa; Old Blackwater; May, ?

Days	1	2	3	4	5	6	7
As. parasites	1,104	—	—	—	1,056	600	400
Crescents	8	—	—	-		4	0
Maximum temp.	—	patient absent			14	12	30
Q. hydrochloride	0	*et seq.*	—	—	—	—	—
Total leucocytes	[illegible]			—	60	120	113
Hb. Tal., percentage	80	—	—	—	70	70	75

Case 28.—J. D., negro fireman, 25 (?); Sierra Leone; Niger: 42 days; June, 151 lbs.

Days	1	2	3	4	5	6	[illegible]
As. parasites	3,040	700	40	136	[illegible]		[illegible]
Crescents	0	*et seq.*	—	—	—		
Maximum temp.	24	38	30	32	18	[illegible]	[illegible]
Q. hydrochloride			.	—	10	[illegible]	[illegible]
Total leucocytes	45	30	70	80	80		[illegible]
Hb. Tal., percentage	—	85	85	[illegible]	85	—	
Ur. urob., mgs.	49	54	0			—	
Faec. urob., mgs.	—	1,130	—	—			—
Days	8	—			—		
As. parasites	0	—	—			—	
Crescents						—	
Maximum temp.	24				—	—	—
Q. hydrochloride	20				.	—	
Total leucocytes	120					—	—
Hb. Tal., percentage	90					—	—
Ur. urob., mgs.	.			.	—		—
Faec. urob., mgs.		—		..	—	—	—

Case 29.—M. S., fireman, 38; Niger; 70 days; June, 149 lbs.

Days	1	2	3	4	5	6	[illegible]
As. parasites	5,300	6,500	3,800	0	0	0	
Crescents	0	4	4	0	0	4	
Maximum temp.	83	69	32	32	32	32	[illegible]
Q. hydrochloride	—	20	30	*et seq.*		—	—
Pol. leucocytes	21	21	22	15	20	28	[illegible]
Mon. leucocytes	12	35	64	18	45	43	[illegible]
Hb. Sal., percentage	60	55	65	65	60	[illegible]	[illegible]
Days	8	9	10	—	—	—	—
As. parasites	0	0	0		—	—	—
Crescents	0	0	0	.	.	—	—
Maximum temp.	32	32	25	—	—	—	—
Q. hydrochloride	—	—	—		—	—	—
Pol. leucocytes	78	45	20	—	—	—	—
Mon. leucocytes...	128	45	30	—	—	—	—
Hb. Sal., percentage . ..	70	65	70	—	—	—	—

Case 30.—J. W., sailor, 53; Niger; 15 days: June, 133 lbs.

Days	1	2	3	4	5	6	7
As. parasites	4,000	2,800	3	8	50	50	12
Crescents	0	0	0	0	0	20	80
Maximum temp.	79	54	22	32	16	18	18
Q. hydrochloride	20	0	*et seq.*	—	—	—	—
Pol. leucocytes	23	22	28	29	22	70	48
Mon. leucocytes	15	85	62	62	30	70	56

Days	8	9	10	11	12	13	14
As. parasites	20	0	0	0	?	0	0
Crescents	120	152	123	116	88	80	72
Maximum temp.	22	22	25	22	29	34	29
Q. hydrochloride	—	—	—	—	—	—	—
Pol. leucocytes	78	35	35	72	42	82	90
Mon. leucocytes	123	60	28	52	50	78	63

Days	15	16	—	—	—	—	—
As. parasites	0	0		—	—	—	—
Crescents	68	56	—	—	—	—	—
Maximum temp.	32	25	—	—	—	—	—
Q. hydrochloride	20	20	—	—	—	—	—
Pol. leucocytes	142	63	—	—	—	—	—
Mon. leucocytes	98	73		—	—	—	—

Case 31.—J. R., sailor, 20; Niger; 90 days; June, 119 lbs.

Days	1	2	3	4	5	6	7
As. parasites	28,000	14,000	25,600	16,800	3,300	1,200	800
Crescents	0	*et seq.*	—	—	—	—	—
Maximum temp.	95	108	101	87	90	54	61
Methylene blue, grs.	—	4	*et seq.*	—	—	—	—
Pol. leucocytes	15	35	25	18	18	15	15
Mon. leucocytes	5	75	25	24	20	30	30
Hb. Tal., percentage		80	85	80	75	80	70

Case 31—*continued*

Days	8	9	10	11	12		—
As. parasites	0	0	0	0	0	—	—
Crescents					—	—	
Maximum temp.	29	25	32	29	32	—	—
Methylene blue, grs.	—	—			—	—	
Pol. leucocytes	15	17	20	65	92	—	—
Mon. leucocytes	38	30	36	135	88	—	-
Hb. Tal., percentage	65	70	75	75	75	—	-

Case 32.—F. H., sailor, 20; W. Africa; 150 days; July. 150 lbs.

Days	1	2	3	4	5	6	[illegible]
As. parasites	60	20	0	0	300	150	[illegible]
Crescents	244	100	108	52	80	104	[illegible]
Maximum temp.	25	22	25	36	29	40	[illegible]
Q. hydrochloride	0	*et seq.*	—	—	—	-	-
Pol. leucocytes	16	28	25	62	60	38	[illegible]
Mon. leucocytes...	25	20	62	125	102	48	[illegible]

Case 33.—Steward, 18; W. Africa; recently; July, 140 lbs.

Days	1	2	3	4	5	6	—
As. parasites	2,500	6,700	50	—	0	0	—
Crescents	0	*et seq.*	—	—	—	—	—
Maximum temp.	108 delirium	61 delirium	87 delirium	69 delirium	40	32	-
Q. hydrobromide	16	10	10	—	-	—	30 *et* [illegible]
Pol. leucocytes	55	35	28	—	35	32	—
Mon. leucocytes...	68	40	22	—	52	58	—
Hb. Tal., percentage	105	95	100	—	100	95	—

A CASE OF BLACKWATER FEVER FOLLOWED BY A PECULIAR RELAPSE WITHOUT HAEMOGLOBINURIA OR DETECTABLE *PLASMODIA*

BY

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This case was studied under the conditions and by the methods referred to in the previous paper, and is, we believe, the first case of blackwater fever which has occurred while daily estimations of the numbers of *Plasmodia* were being made by thick film methods. Though there is much evidence to connect blackwater fever with malaria, yet many authors doubt whether the typical fever generally associated with the haemoglobinuria is due entirely or at all to the parasites of malaria. Our case is of special interest because it throws considerable light on this question. It will be seen from the chart that the case had three separate pyrexial periods while under observation; that the first of these was an ordinary malarial attack; the second an ordinary blackwater attack (without detectable parasites); and the third a singular attack during which neither blackwater nor parasites were found—strongly suggesting, if not proving, that the later attacks were due to some cause other than the toxin of the *Plasmodia*, and that haemoglobinuria may be merely an occasional result of this unknown cause. A fourth attack occurred still later but, unfortunately, could not be studied by us.

The patient, A. E. B., male, aged 32, an English trader on the Gold Coast since 1903, stated that he had slight fever during his first year in West Africa, and numerous slight or severe attacks since then, but without any observed blackwater fever. He did not take much quinine until more recently, when (in February, 1910) he was given 30 grains daily for a fortnight, without its producing blackwater. He was admitted to the Royal Southern Hospital, Liverpool, on the 2nd June, 1910, owing to the continuance of attacks. His weight was 114 pounds (52 kilograms); he looked pale, thin and anaemic; his liver was enlarged half an inch below the costal margin, but his spleen, though enlarged, was scarcely palpable.

As shown on the chart, on the day of admission 2,000 asexual forms of *Plasmodium falciparum* were found per c.mm. of his blood accompanied by the usual fever. On the following five days the same parasites numbered 80, 1,360, 880, 20, and 12 per c.mm., accompanied by fever on the 2nd, 3rd, and 4th days after admission. Quinine hydrochloride (10 grains) was given on the 4th day and 30 grains daily until the 10th day inclusive. No parasites were found on the 7th and 8th days, and no fever occurred on the 5th, 6th, 7th and 8th days. On the 7th and 8th days he was allowed out of hospital for a few hours in the afternoon, and on the latter day got heated with exercise, and ate ices.

On the 9th day he was attacked with high fever with rigors. On the 10th day haemoglobinuria commenced and the quinine was stopped. The attack was very serious. The haemoglobinuria cleared up on the evening of the 11th day, but the fever continued to a diminishing degree until the 18th day, though the temperature occasionally rose slightly above normal until the 37th day. There was then a break for four days, on the last of which he was again allowed out of hospital. On the 42nd day another severe pyrexial period commenced, lasting until the 54th day, but not accompanied by any haemoglobinuria. The patient now improved greatly and was discharged on the 63rd day; but returned again to hospital on the 76th day with fever (which, however, was not examined by us), but no blackwater. The parasites were carefully searched for (by D. T.), both in thick and thin films, on forty days up to the 49th day of the disease, after which, unfortunately, the examinations could not be continued. On not a single occasion since the 6th day of the disease could a single *Plasmodium* be found, though during the 9th, 10th and 11th days, that is, during the blackwater period, the blood was examined by both methods four times daily. No crescents were ever seen.

It should be noted that the haemoglobin of the blood, estimated (by D. T.) by Sahli's haemoglobinometer, began to fall on the day, the day before the fever of the 9th day commenced, and fell 10 per cent. daily during the next four days—the total fall being from 75 to 25 per cent. during seven days. It remained at the latter figure and then rose by about 5 per cent. daily for four days, and then more slowly. A slight fall interrupted the continuous rise on the 29th day, but on the 40th day the haemoglobin had reached 90 per cent. During the second relapse, occurring on the 42nd day, it underwent a total fall of only 12 per cent.

The red cells varied as follows:—

Days	11	12	14	18	20	21	22	25	29
Numbers in thousands	2,600	1,150	800	1,450	1,600	1,900	2,000	2,500	2,800

Regarding the urobilin (G. C. E. S.), there was only a very slight rise in the urinary excretion, reaching a maximum of 63 mgms. during the first febrile period. During this time two observations of the faecal excretion disclosed a total of 1,830 mgms. Commencing from the 10th day the faeces were examined continuously, and disclosed a urobilin excretion of 32,500 mgms. during six days, falling after this to the low figure of 100 mgms. per diem. During the blackwater period there was a marked degree of urinary urobilin, namely, 340 mgms. on the 10th day, 300 mgms. on the 11th day and 115 mgms. on the 12th day—after which it fell to the low level of 30 to 60 mgms. a day. The actual quantity of haemoglobin in the urine was estimated in each sample passed during the period of blackwater, and reached its height at 7 p.m. on the 10th day, and then fell gradually as follows:

10th Day

Hours	10 a.m.	7 p.m.	11 p.m.
Quantity of urine	95 c.c.	90 c.c.	200 c.c.
Amount of Hbg. (expressed as red blood cells by volume) ...	Trace	4·5 c.c.	0·5 c.c.

11th Day

Hours	2 a.m.	9 a.m.	12 noon	7 p.m.	9 p.m.
Quantity of urine	250 c.c.	110	200	625	360 c.c.
Amount of Hbg. (expressed as red blood cells by volume)	0·625 c.c.	0·055 c.c.	0·33 c.c.	0·	1·98 c.c.

12th Day

Hour	3.30 a.m.
Quantity of urine	570 c.c.
Amount of Hbg. (expressed as red blood cells by volume)	0·

In all, the haemoglobin of 17 c.c. of normal blood or about [illegible] grams of haemoglobin was passed unaltered in the urine.

We should note that during the blackwater period and subsequent fever up to the 23rd day, chlorides were markedly diminished in the urine and the specific gravity was rather low.

During the febrile periods beginning on the 9th and on the 42nd days there was no scarcity of leucocytes such as occurred during the first febrile period, or such as occurs during ordinary malarial febrile periods; but the mononuclear percentage was high all through, as occurs during malarial fever and afterwards. Much bilious vomiting occurred on the 10th and 11th days, and unaltered bile pigment was present in the faeces up to the 15th day. The patient had the usual jaundiced appearance during the second febrile period.

Quinine, which had been stopped since the 10th day, was resumed in doses of one grain daily on the 35th day and two grains daily from the 41st to the 46th day, when it was stopped again on account of the second relapse. The urobilin was not estimated during the third pyrexial period.

On studying the case, we observe that the first pyrexial period from the 1st to the 4th day accorded exactly with our previous experiences with the thirty-three ordinary cases of malaria described in our first paper ('Enumerative Studies on Malaria'). The amount and type of fever, number of leucocytes, fall of haemoglobin, amount of urobilin excretion, and effect of quinine were precisely what we would have expected from the number of parasites counted; and the same thing may be said of the following apyrexial period. But the subsequent events differed markedly from our previous experiences. Although no parasites whatever were found in numbers of thick film preparations, yet the fever, fall of haemoglobin, and urobilin excretion in the later febrile periods were so severe that they would usually be associated with, say, 50,000 parasites or more per c.mm. The type of fever in these periods also differed from that of the first period and from that seen in our cases of malaria. During the second period (of the 9th day) the fever consisted of several severe paroxysms on each day, accompanied by sharp rigors and profuse sweats all of these much more severe and continued than are usually seen in old cases of malaria, even with very many parasites. The third pyrexial period was very similar to the second one, except only that it was not so severe, and that haemoglobinuria was entirely absent. Moreover, the whole comparatively fever-free period from the 18th to the 41st day, in which the temperature frequently exceeded normal, differed from ordinary malarial apyrexial periods, in which the temperature generally remains quite and even considerably below normal, and asexual parasites are often still to be found. Quinine certainly did not prevent the second and third pyrexial periods, although it was never followed by relapses in our cases of malaria; and the leucocytes did not show the characteristic diminution of malaria.

From these facts we are inclined to infer, almost with certainty, that the second and third pyrexial periods were not due to the toxin of the parasites of malaria, that is, to the usual cause of malarial attacks, but to some other cause. As shown in our previous paper, the fact that parasites were not found in no less than forty-three thick film specimens does not prove by any means that none were present in the whole body; but it proves that their numbers were much below the usual figure required to produce even slight fever. If, then, the later pyrexial periods were due to the small remainder of parasites, either the 'virulence' of these must have become suddenly and greatly exalted after the 9th day, or else the patient's resistance to their toxin must have become suddenly lowered. Neither of these appears probable, because the general type of the fever in the later periods differed so much from the usual malarial type, and because quinine had no reducing effect. If there is another cause, as seems to be highly probable from this case, it must exert a powerful haemolytic as well as pyrogenous effect. The third pyrexial period is of great interest, because it appears to establish a new fact, namely, that this peculiar fever, though generally associated with a great fall of haemoglobin and also with haemoglobinuria, need not necessarily be associated with either (the *Plasmodia* being at the same time undetectable). This proves also that mere haemoglobinuria by itself does not cause the fever.

We now content ourselves with merely recording the observed facts without discussing the possible causes of the fever. Further researches are required to ascertain whether similar phenomena occur in other cases. It has been often shown since Panse's* researches that the parasites tend to disappear at the onset of blackwater are not, we think, so conclusively as in this case; nor do we know relapses without either *Plasmodia* or haemoblobinuria having been previously noticed.

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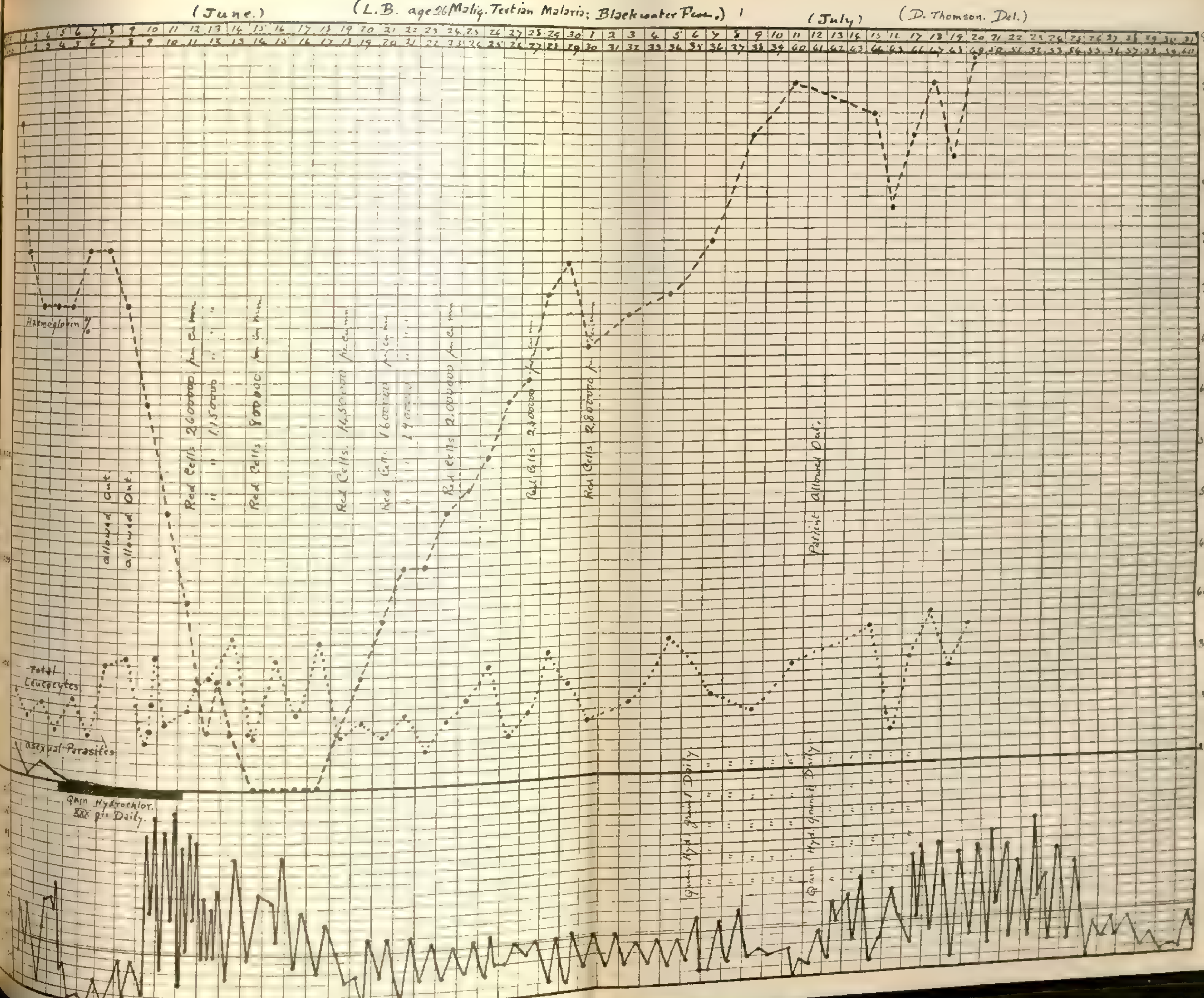
* Otto Panse. Zeit. für Hygiene, 1902.

We should add (1) that no signs of any other parasites besides *Plasmodia* were seen; and (2) that the total elimination of urobilin from the 8th to the 15th day represents an amount of haemoglobin equal to that of *all* the patient's corpuscles, the 25 per cent of haemoglobin still remaining being probably contained in young corpuscles generated during the period referred to. The relation between the numbers of circulating corpuscles and the haemoglobin percentage appears to confirm this estimate. As the asexual parasites cannot live outside the red corpuscles, this enormous destruction of the latter probably causes an equally great destruction of the former, which may explain Panse's law and the absence of parasites after the sixth day in our case; but it does not seem capable of explaining the fever. (3) The comparatively slight fall of haemoglobin during the third pyrexial period may be explicable on the supposition that the red corpuscles were then probably younger and more resistant. (4) The enlargement of the liver and the bilious vomiting might suggest derangement of the hepatic system, but they may also represent the hyperaemia of extreme activity. The fact that the liver eliminated, in its usual manner, such an enormous amount of blood pigment tends to confirm the latter hypothesis. (5) In conclusion, we would remark that haemoglobinuria seems to be merely a small overflow of the freed haemoglobin which the liver has not been able to deal with; and that some other cause other than the toxin of the *Plasmodia* seems to produce both the specia haemolysis and the special fever associated with it.


(June.)
(L.B. age 26 Malig. Tertian Malaria; Blackwater Fever.)
(July)
(D. Thomson. Del.)
Haemoglobin %
allowed Out.
allowed Out.
Red Cells 2600000 per cu mm
" " 1150000 " " "
Red Cells 800000 per cu mm
Red Cells. 1450000 per cu mm
Red Cells 1600000 per cu mm
" " 1400000 " " "
Red Cells 2000000 per cu mm
Red Cells 2300000 per cu mm
Red Cells 2800000 per cu mm
Patient allowed Out.
Total Leucocytes
asexual Parasites
Quin Hydrochlor. XXX grs Daily.
Quin. Hyd. grain 1 Daily
Quin Hyd. grain ii Daily
95%
90%
85%
80%
75%
70%
65%
60%
55%
50%
45%
60%
35%
30%
25%
Percentage of Haemoglobin (Sahli)

ON HAEMOGLOBIN METABOLISM IN MALARIAL FEVER

BY

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I. INTRODUCTION

The following studies were carried out at the suggestion of Major Ross on some of the thirty-three cases of malaria which have occurred since January, 1910, at the Royal Southern Hospital, Liverpool, and which have already been reported upon by him and Dr. David Thomson in their paper on 'Enumerative Studies on Malarial Fever.'† The funds for the research were allotted to the Liverpool School of Tropical Medicine by the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office). Much of the work was done in the laboratory of Professor Benjamin Moore, University of Liverpool, to whom we are indebted for advice and assistance.

The object of the research was to ascertain the fate of the haemoglobin, which is well known to be set free from the red corpuscles in malarial fever, and to measure its amount, in the ultimate hope that more light might thus be thrown on various problems connected with malaria, and especially with blackwater fever. The daily details of my findings are incorporated in the tables of the paper just mentioned, in order that they may be read in connection with the marked fall, recorded by the haemoglobinometer, in the haemoglobin of the circulating blood during the pyrexial periods.

II. PRELIMINARY

At first a careful search of the urine was made during and after the malarial paroxysms in eight patients. No trace of haemoglobin or of haemin or other immediate derivatives of haemoglobin was found, except in two cases, where a few red blood corpuscles were found associated with oxaluria. Hyaline and granular casts and oxalate crystals were present in the urine for a few days after the paroxysms in five of these cases (Nos. 10 to 14 inclusive. A greater measure of success was attained when attention was directed to the more remote products of haemoglobin breakdown, of these the iron-free pigment nucleus alone held out prospects of successful quantitative work, since the iron itself is known to be in a large measure retained in the body and the protein portion is merged in the general protein metabolism. Haemoglobin, whether arising by haemolysis in the body or experimentally introduced, is withdrawn from the circulation and broken down into its constituents by the liver, and the pigment moiety is excreted into the intestine as bile pigment. When amounts larger than about 0·3 gram of haemoglobin per kilo body weight are suddenly introduced into the circulating blood, some part may be excreted by the kidneys, and Barratt and Yorke* have shown that the haemoglobinuria varies with the degree of haemoglobinaemia.

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* (Read in abstract before the Royal Society, Dec. 8, and reprinted from Proc. Roy. Soc., Series B., Vol. LXXXIII. p. 174.)

† Annals of Trop. Med. and Parasit., Vol. IV, No. 3, p. 267.

The bile pigment leaves the body for the most part as urobilin in the urine and the faeces.

Since the relationship of haemoglobin and urobilin is known with fair accuracy, it is possible to calculate theoretically the amount of urobilin which would be yielded, if all the haemoglobin of a normal man could be transformed into that substance; or conversely what amount of haemoglobin must have been destroyed to give rise to a known excretion of urobilin. A man of 60 kilogrammes with a normal amount of red corpuscles, etc., would yield about 30 grams of urobilin from the haemoglobin (750 grams) of the circulating blood and about 20 grams more from the haemoglobin of his muscle and from pigment in the liver and bile passages.

III. URINARY UROBILIN

(*a*) The work of Hoppe-Seyler, Hildebrandt, and others has shown that urobilin is present only in traces in normal urines, but that it is increased in pyrexia, anaemias, hepatic disorders and internal haemorrhage. The excretion is from 0·01 to 0·1 gram per diem in the urine of healthy adults, and in pyrexias, etc., it rises as high as 0·5 gram per diem. Occasionally higher figures have been recorded, 0·8 gram in scarlet fever, 2·02 gram in perityphlitis and 2 grams in malaria.

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* Annals of Trop. Med. and Parasit., Vol. III, No. 1, Section V.

After trials of various methods, the method of quantitative determination adopted for the purpose of this work was to determine the dilution of the urine required to just render invisible the spectrum of urobilin, when examined by a spectroscope in a layer of 15 mm. thick. The spectroscope was standardised with solutions of purified urobilin, and the readings appear to be reasonably accurate except that, when only a low concentration of urobilin is present, they are too low. The spectroscopic results were checked in many instances by the more delicate fluorescence tests.

By this method I failed after repeated trials to find urobilin in any specimen of urine from healthy adults; amounts varying from 0·04 to 0·25 grams per diem were found in various pyrexial diseases and from 0·02 to 0·06 gram per diem in cases of anaemia, intermuscular haemorrhage and hepatic disease. In a case of Malta fever (temp. 104° F.) and in a case of acute suppurative hepatitis (probably dysenteric) (temp. 104° F.) 0·3 and 0·31 gram per diem were found respectively.

(*b*) In five cases of *simple tertian malaria* (*P. vivax*) the amount of urobilin found in the urine in the period of pyrexia and the days following was never greater than the average excretion in diseases such as pneumonia with an equal degree of pyrexia, and in Case 3, indeed, was scarcely perceptibly increased from the normal limits:—

Maximal excretion in twenty-four hours (Case 4) 0·19 gram.

Maximal total excretion in seven days after pyrexia (Case 4) 0·42 gram.

(*c*) *In malignant tertian malaria* (*P. falciparum*) a different picture is obtained; definite urobilinuria sets in very shortly after the onset of the pyrexia, reaches its height on the second or third day and then slowly diminishes; the normal level of excretion is reached in seven to ten days unless a fresh paroxysm intervenes. Sometimes the increased output continues for a longer period (*e.g.*, Case 20), and in old standing cases with chronic malarial anaemia there may be a continuous raised urobilin output even in the absence of pyrexia (Case 19); and in these cases marked haematoporphyrinuria was several times noted (Cases 17, 18, 19). (Haematoporphyrinuria was also noted on one occasion in Cases 20 and 23.)

Average excretions of urinary urobilin in malignant tertian malaria (Cases 10, 12, 17, 19, 20).

Maximum excretion in twenty-four hours (Cases 20 and 17) 0·2 to 0·30 grams.

Maximum total excretion in seven days (Cases 17 and 12) 0·6 to 0·7 gram.

The amount of urobilinuria (even if considered in comparison with body weight) bore no apparent relation (1) to the degree of pyrexia; (2) to the fall of the haemoglobinometer readings during the pyrexia; or (3) to the absolute numbers of parasites present in the blood.

(*d*) *Special high excretion in malignant tertian malaria.* In four cases the excretion of urobilin was so much higher that they will be mentioned separately and in more detail. In Cases 11, 13, and 23, the patients were young, and large numbers of parasites were present with correspondingly severe symptoms; in Case 21 the patient was older and, though the parasites were much less numerous and the pyrexia less marked, he was markedly prostrated by his attack.

I may mention that the urine in these cases became extremely dark on standing in daylight, after acidification, and that the band of urobilin remained visible in two instances until the urine was diluted to one hundred and fifty volumes. Cases 13 and 21 were constipated at the time the high-days' output took place.

I give in each instance the maximal twenty-four hours' output and the total excretion for seven days, and have also determined the percentage relation between these amounts and the normal circulating haemoglobin of the patients.

Number of Case	11	13	21	23
Weight in kilos.	60	54	72	50
Maximal 24 hours' excretion—				
As grams of Urobilin	2·31	0·9	1·73	2·0
As percentage of total Hbg.	8·1	3·5	5·0	9·5
Total 7 days' excretion—				
As grams of Urobilin	3·9	1·7	2·38	2·26
As percentage of total Hbg.	14·0	6·8	7·2	11·2
As corpuscles per $mm.^3$ of blood	700,000	340,000	360,000	560,000
Fall in Haemoglobin as recorded by Haemoglobinometer	?	?	30 %	20 %
Number of parasites per $mm.^3$ of blood	64,000	300,000	36,000	50,000

The figures are very high indeed, and equal, in some instances, any figure that I have found recorded by other observers; their special interest is that they show the destruction and elimination of a very large proportion of the haemoglobin of the circulating blood; I propose, however, to leave the question of urinary urobilin at this stage and proceed to consider the elimination by the bowel. As will be seen later urinary urobilin would appear to be simply a leakage—rarely an important one—from the other channel, and it has only been alluded to in consideration of the great attention that has been paid to it in the past, and since in the present instance, it was largely the promise held out by the results obtained in Cases 11 and 13 in the early days of January, that led to my persisting in further work along these lines.

Note.—Since writing this paper I have become acquainted with De Jonge's* results, he found urobilin in malarial urines in amounts of from 0·05 to 0·10 gram per diem, and this frequently rose as high as 1 gram and occasionally even to 4 grams. The difference may be accounted for by the fact that his cases, unlike mine, were frequently treated with quinine, which he found to increase the urobilin output. He found however lower outputs in malignant tertian malaria than in benign tertian, a result very different from mine.

IV. FAECAL UROBILIN

(*a*) It would be out of place in the present paper to discuss at any length the methods of extraction and estimation of urobilin from the faeces, but perhaps I may be pardoned for alluding to a few points of interest.

After considerable controversy it has been shown that urobilin of identical quantitative composition can, by various processes, be prepared from faecal and urinary sources. It would appear, however, that much of the urobilin in the faeces is present in modified forms (possibly in part in union with metallic salts) since it has a fluorescence and occasionally a spectroscopical appearance somewhat different from that of urinary urobilin; on one or two occasions I was very much puzzled by obtaining a solution much more purple in colour than usual, and with a slightly altered band, and it was only after considerable expenditure of time that I discovered that part of the urobilin was present as its mercury compound owing to the administration of calomel.

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* Geneesk. Tijdschr. v. Nederl.-Indie, Deel XLIV, p. 435, 1904.

Another variation which gave rise to great difficulty is that the urobilin, obtained by the extraction of the faeces with acidulated water, proves very refractory at different stages in the process of purification and separation, described by Garrod and Hopkins—indeed we have as yet failed to get a reasonably pure product from the faeces and so possibly the figures obtained may later require revision.

The urobilin was extracted by repeated shaking with large amounts of water, acidulated with dilute sulphuric acid, and the filtrates were fully exposed to daylight for some time, before being estimated by the same method as was adopted for urinary urobilin; this solvent has the advantage that it does not take up bile pigment or chlorophyll to any extent, and only on one occasion (later extracts of a case of blackwater fever) did I find any definite band other than that of urobilin, though other writers seem to have found such extra bands very frequently when using different solvents.

Another point of interest is that in such literature as I have discovered on faecal urobilin, the writers seem to convey the impression that it is present in small amounts and requires somewhat delicate tests for its identification. Only in McMunn's papers did I find descriptions of solutions comparable in colour and spectroscopic appearances to those with which I had become acquainted, and in which, as a general rule, a very high dilution was necessary before the general absorption was overcome; and the urobilin band was the only one ever distinguished save in the instance referred to in the last paragraph, when a band was also present embracing the D line, probably due to haematoporphyrin.

From Case 23, for example, nearly two litres of acidulated extract were obtained in which there were visible:—

1. Acid Urobilin Band	to a dilution of	100	volumes.
2. Mercury "	" ...	120	"
3. Pink colour of mercury urobilin	" ...	800	"
4. Fluorescence with Zinc Chloride	" ...	300	"

In another instance (blackwater fever case) from the same stool I obtained:—

4,000 c.c. of extract with Acid Urobilin band visible to 400 dilutions and Fluorescence (Zinc Acetate) to 1,600 dilutions.

7,400 c.c. of extract with Acid Urobilin band visible to 200 dilutions and Fluorescence (Zinc Acetate) to 1,000 dilutions.

3,000 c.c. of extract with Acid Urobilin band visible to 40 dilutions and Fluorescence (Zinc Acetate) to 600 dilutions.

In all 3,200 litres of fluid showing Urobilin band, or 15,600 litres of fluid showing clearly green Fluorescence with Zinc Acetate, in each case in test-tubes fifteen millimetres in diameter.

The only quantitative figures I have found in the literature for faecal urobilin are:—

0·021 to 0·029 gram per diem in cases of Chlorosis.
0·153 to 0·92 ,, ,, ,, Pernicious Anaemia.

My own results included the following:—

Stools of healthy adults on full diet ...	0·03 to 0·06	gram	per diem.
Pneumonia (temp. 104°F., milk diet) ...	0·08	,,	,,
Malta Fever (temp. 104°F., milk diet) ...	0·4	,,	,,
Anaemic Girl (meat diet) ...	0·04 to 0·13	,,	,,
Intramuscular Haemorrhage (meat diet)	0·12 to 0·13	,,	,,
Amoebic Suppurative Hepatitis (temp. 103°) ...	0·12 to 0·13	,,	,,
Chronic Malarial Anaemia	0·15	,,	,,

(*b*) *Faecal urobilin in benign tertian malaria* (*P. vivax*). In three cases of this disease the excretion was found to be comparable to that found in other pyrexial diseases (in a fourth, Case 8, it was much higher, but was only taken on one day).

Case 6—Maximum day's excretion	0·49 gram.
Total excretion in 7 days	1·22 ,,
Control stool (13th day, meat diet)	0·02 ,,
Case 7—Maximum day's excretion	0·53 ,,
Total excretion in 7 days	1·30 ,,
Control stools (10th and 11th days, meat diet) ...	0·07 and 0·05 gram.
Case 8—Only one stool examined (4th day)	1·12 gram.

These excretions represent in Cases 6 and 7 about 4 per cent. of the patients' total circulating haemoglobin, the haemoglobinometer shows a much greater fall (20 per cent. in Case 7).

(*c*) *Faecal urobilin in malignant tertian malaria* (*P. falciparum*). Eight cases of disease have been examined for faecal urobilin, and in practically every instance a higher output was shown.

In five instances, only isolated stools shortly after the paroxysm were examined, and usually also a control stool, when the patient was again convalescent and on meat diet.

Case Number	Day of disease	Faecal Ub. (grams)	Faecal Ub. (per cent. of Hbg.)	Fall of Haem. (Haemoglobinometer)
17	4	1·13	4	20
	13 (relapse)	0·45	1·6	10
	21 (control)	0·28	1·0	—
24	20 (relapse)	0·8	2·4	5
	16 (control)	0·14	0·42	—
25	1	0·12	—	—
	7	0·51	—	—
28	2	1·13	3·5	5

In the two remaining cases the faecal excretion was followed throughout the attack, save for occasional unavoidable losses. The history of these cases (21 and 23) has already been alluded to in the section dealing with urinary urobilin.

In Case 23 the urobilin output during convalescence and on meat diet was from 0·01 to 0·02 gram per diem, and during his pyrexia this rose to as much as 1·73 grams.

In the first seven days after the onset of pyrexia his total urobilin output was 6·4 grams, representing no less than 25 per cent. of his total haemoglobin (of this amount 4·3 grams appeared in the faeces, 2·1 grams in the urine). His haemoglobinometer reading fell 20 per cent. and recovered 6 per cent. in the last two days of the week; the number of red blood cells destroyed (calculated from urobilin excretion) must have been about 1,250,000 per mm.^{3}, while the maximum number of parasites observed was only 50,000 per mm.^{3}.

In two subsequent relapses his urobilin output was very much lower (about 1·5 grams), but in the first of these especially a much more marked fall occurred, as shown by the haemoglobinometer, and the recovery was much slower, suggesting possibly that the reserves of haemoglobin precursors had become exhausted.

Case 21 had a rather higher output of urobilin during convalescence, amounting to 0·15 gram per diem, but after pyrexia it reached the high figure of 4·1 grams (following three days' constipation). Some stools were unfortunately lost, but his total urobilin output (urinary and faecal) amounted to 6·8 grams, which represents 20 per cent. of his total normal haemoglobin.

The haemoglobinometer readings fell 25 per cent., of which 15 per cent. was regained within a week. His urobilin output corresponds to a destruction of 1,000,000 corpuscles per mm.^{3}, while the maximum number of parasites seen in the peripheral blood was 35,000 per mm.^{3}.

V. SUMMARY

There can therefore be little doubt that, in malignant tertian (*P. falciparum*) infection, the excretion of haemoglobin derivatives is much higher than in other diseases with a similar degree of pyrexia; this excretion is accompanied by, and probably depends on, a fall in the haemoglobin present in the circulating blood, and would seem to demonstrate that the fall in the haemoglobinometer readings represents an actual destruction of red blood cells, not merely an alteration in the relative proportions of corpuscles and plasma in the peripheral blood.

In Cases 21 and 23, respectively, thirty-five and twenty-five corpuscles at least must have been destroyed for every parasite present, and this suggests either that the corpuscles are disintegrated mechanically (or as part of a protective mechanism) by the efforts of the spores to enter them, or else that the mother-parasite contains some haemolytic substance which is liberated on sporulation.

Finally I would like to draw attention to the relationship between the elimination by the urinary and faecal channels in these cases, and, indeed, in health and in other diseases. Only exceptionally does the urinary output approach the level of the faecal output, and yet practically all the quantitative work that has so far been done on urobilin, has been confined to the small and comparatively unimportant channel.

Before concluding I would like to refer to the eighth case of malignant tertian infection mentioned in dealing with faecal urobilin, and which, owing to its peculiar interest, is dealt with in a separate paper by Major Ross, Dr. Thomson and myself (p. 307).

When admitted to the hospital he had a fairly severe attack of malignant tertian malaria, and in the week following its onset he eliminated about 3 grams of urobilin, the large majority in the faeces; this is equivalent to 10 per cent. of his total circulating haemoglobin, and corresponds with the elimination in ordinary cases of this infection; (under normal circumstances on full diet his faecal urobilin is about 0·18 gram per diem).

A week later he had another attack of fever, accompanied by haemoglobinuria—the complex known as blackwater fever—and in the first seven days of this attack he excreted the enormous amount of 33 grams of urobilin. In spite of marked urobilinuria during the period of haemoglobinuria only 1·25 grams of urobilin appeared in all in the urine, and of the remainder nearly the whole (29·3 grams) was present in one stool, of which some particulars were given in the preliminary remarks on faecal urobilin (this stool contained also a considerable amount of unaltered bile) Owing to his collapsed and unconscious state, several stools were lost, but his next three recoverable stools contained in all only some 0·8 gram of urobilin, (though the presence of diarrhoea may have led to an unduly low yield).

As will be remembered, a man of his weight (60 kilos) contains potentially some 30 grams from the haemoglobin of his circulating blood and probably about 20 grams more from other sources, so this patient seems to have suffered an almost total destruction of his circulating blood. The haemoglobinometer reading fell to 25 per cent., and so apparently some of the destroyed haemoglobin was rapidly replaced from other sources. The haemoglobinuria itself only accounted for the haemoglobin of 20 c.c. of blood, less than 0·5 per cent. of the total.

I do not propose to allude further to this case, but would like to indicate one other possible point of interest. Barratt and Yorke* state that haemoglobinuria arises in man, if a grade of haemoglobinaemia is attained exceeding that obtained by the solution of the haemoglobin of 0·25 per cent. by volume of wet red cells in the blood plasma; (roughly speaking, the volumes of plasma and corpuscles are equal in human blood, and so on this scale total haemolysis represents 100 per cent).

We have seen that in Cases 21 and 23 from 20 to 25 per cent. of the corpuscles were destroyed, and in these cases the period of pyrexia

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* *Loc. cit.*, Section III.

(in which it appears legitimate to assume the haemolysis took place), occupied about thirty-six hours. So the haemoglobin of about 0·6 per cent. of red corpuscles was discharged into the plasma in every hour, if the haemolysis was spread evenly over the period of the crisis; and as the liver would need to remove this amount every hour from the whole blood to prevent the appearance of haemoglobinuria, any slight failure of the mechanism either from hepatic congestion or from all the haemolysis occurring in a very short period of time might serve to set up haemoglobinuria.

CONCLUSIONS

1. The main excretory channel for the pigment portion of the blood is the alimentary tract, and urinary urobilin represents only a small overflow from this source; the main elimination is in the faeces, and this has been largely neglected by previous observers, in favour of the urinary overflow; which, however, would seem to be of importance only as an indication of the absorptive activity of the intestine and almost negligible for quantitative purposes.

2. That the fall in the haemoglobinometer readings in malaria appears to represent an actual destruction of red corpuscles and elimination of their pigment by the normal excretory mechanism.

3. The haemoglobin breakdown during the pyrexia due to malignant tertian (*P. falciparum*) infection appears to be greater than in other pyrexial diseases or than in benign malaria (*P. vivax*), and may be many times greater than would be accounted for by the number of corpuscles infected by the parasites.

4. That as regards their effect of causing haemolysis, the parasites appear to be of different degrees of virulence in different individuals, and even in different paroxysms in the same individual (relatively at least, since the patent's resistance must be considered).

5. Normally the ordinary channels of excretion are capable of dealing with the free haemoglobin and rapidly remove it from the blood stream, and a very severe strain (over 25 per cent. of the total circulatory haemoglobin) can be sustained without their failure and the consequent onset of haemoglobinuria.

SOME OBSERVATIONS ON A CASE OF SLEEPING SICKNESS: COAGULATION TIME OF BLOOD, ALBUMOSES, CHOLINE, CEREBRAL SECTIONS

BY

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INTRODUCTORY REMARKS

The observations recorded in this paper, though somewhat incomplete, are not without interest. They were made on a case of sleeping sickness in Professor Ross's clinic at the Royal Southern Hospital, Liverpool. This work was done in conjunction with other researches on the same case.*

Patient, W. A., male, age 26, was infected by the parasite of sleeping sickness in September, 1909, while he was in North East Rhodesia. Trypanosomes were observed in his peripheral blood in Africa on November 17, 1909. He died of lung and pleural complications (pneumonia and empyema) on June 29, 1910. The exact period of disease may be approximately calculated to be 300 days or thereabouts. The somnolent stage was not well pronounced. The parasite found in his blood showed a peculiar morphological feature in that some of the stout forms possessed a posterior nucleus, and has been named *T. rhodesiense* by Stephens and Fantham.† This parasite was of a marked virulence in the laboratory animals. Such in brief is the history and peculiarities of the case.

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* Ross and Thomson. Proc. Roy. Soc., 82 B, pp. 411-415.

† Stephens and Fantham. Proc. Roy. Soc., 83 B, pp. 28-33 (1910).

I. *Coagulation time of blood, by Wright's method at* 37°C. (*blood heat*).

In order to understand the relations between the coagulation, leucocytes, haemoglobin and the parasites, the observations are tabulated below. They were made within the last fortnight of the patient's illness.

	Normal (Man)	June 14	June 16	June 21	June 29
Coagulation time ...	3 to 6 minutes (4 mins. 30 secs. average)	12′ 0′	5′ 25″	5′ 0	Died
Leucocytes per mm.^{3}	10,000	25,000	4,500	3,200	
Haemoglobin index (Sahli's scale) ...	100 %	50 %	70 %	50 %	
* Parasites per mm.^{3}	—	38	32	56	

The observations are too few to lead to any definite conclusions, but one gets a general impression that the coagulation time is passing from the negative to the positive phase as the leucocytosis is passing towards leucopenia. The number of the parasites and the nature of the haemoglobin index are subject to very little variation.

II. *Absence of trypanosomes from the cerebrospinal fluid and blood.*

Patient died June 29th at about 5 p.m. Post-mortem made June 30th at about 11 a.m.

(1) The cerebrospinal fluid was carefully collected in sterilized tubes. It was mixed with a trace of blood and lymph (oedema of the brain).

(2) Tubes were centrifugalised for fifteen minutes, the supernatant fluid was pipetted off, smears were made, both from the deposits and supernatant fluid, fixed in alcohol, stained according to the Romanowsky method. The result was negative.

(3) Smears were made from the blood, fixed in alcohol stained with Romanowsky. Result, negative.

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* Counted by Dr. David Thomson.

III. *Presence of albumoses in the cerebrospinal fluid.*

(1) Acetic acid and saturated sodium chloride reactions—positive.*

(2) Biuret reaction—positive.

Besides it appeared from the methodical application of other tests that the albumose belonged to the class of primary proteose.

IV. *The blood was examined for the presence of albumoses. The result was negative.*

V. *Absence of albumoses in the urine.*

The urine during life was examined for albumose. The reaction was negative; later on, in the preserved specimen of urine albumose was again found to be absent.

Patient had a large collection of pus in the pleural cavity, consequently the possibility that the albumose was due to empyema was thus eliminated by the result of the blood and urine examinations.

So that although the cerebrospinal fluid was tinged with blood, the process of exclusion leads to the conclusion that albumose was present in the cerebrospinal fluid only. According to Byrom Bramwell† the presence of albumins, together with lymphocytosis in the cerebrospinal fluid, are supposed to be of early diagnostic value in the incipient stage of parasyphilitic lesions like tabes and general paralysis of the insane. The exact origin of albumose is doubtful; either it may be due to chronic meningo-encephalitis or to toxic products of protozoa or bacteria. Whatever the cause may be it is evident that the presence of albumose is not restricted to the parasyphilitic lesions only.

Its presence in the cerebrospinal fluid in the *incipient* stage of chronic meningo-encephalitis (*T. gambiense*) requires to be determined. But how far it may be of diagnostic value in the case of sleeping sickness is another question.

VI. *Presence of choline.*

Mott and Halliburton have shown that in general paralysis of the insane the marked degeneration that occurs in the brain is accompanied by passing of the products of degeneration into the cerebrospinal fluid, and of these nucleo-proteid and choline (decomposition product of lecithin) are those which can be most readily detected.

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* Boston, Clinical Diagnosis (1904), p. 216.

† Personal communication.

This is not peculiar to general paralysis of the insane, but choline can be detected in various other nerve degenerations.

The point of peculiar interest is that the pathological changes in general paralysis of the insane and sleeping sickness are more or less similar. The test used for choline was that of Mott and Halliburton, viz., the production of characteristic octohedral crystals of the platinum double salt from the alcoholic extracts of the cerebrospinal fluid. The result was positive.

Control tests were made with the 15 per cent. alcohol and absolute alcohol used in the original test, but these were negative.

VII. *Blood.*

With the above test it was found that choline was absent in the blood.

VIII. *Absence of choline in the blood of animals infected with the parasite of the case.*

The test applied was the modified method of Mott.* It consisted in the formation of the brownish-black rectangular plates of *choline periodide.*

The number of the trypanosomes per mm.^{3} at the time of the experiment and before death of the animals are given below.

	Trypanosomes at the time of the experiment	Trypanosomes before death
Rat 1.—Weight 182 grams	20,085 per mm.^{3}	126,000 per mm.^{3}
,, 2.—Weight 220 grams	56,320 ,,	100,400 ,,
Guinea-Pig 1.—952 grams	3,328 ,,	6,000 ,,
,, 2.—880 grams	4,416 ,,	30,720 ,,
Two control rats	0	0

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* *Lancet*, July 9, 1910, p. 80.

Thus choline was absent in the blood practically at the height of the parasite infection.

According to Plimmer* the brains of rats that have been inoculated with the human trypanosomes have shown the peculiar perivascular infiltration. It is interesting to note from my observations that choline failed to enter into the blood, or in other words the products of nerve degeneration have not passed into the blood.

IX. *Effect of products of nerve degeneration in the cerebrospinal fluid (i.e., choline and nucleo-proteid) from the case of sleeping sickness—on the coagulation time of blood and the percentage of haemoglobin in the rat.*

	Before inoculation of cerebrospinal fluid intraperitoneally	After inoculation of the same intraperitoneally				
		1st day	3rd day	4th day	5th day	6th day
	min. sec. min. sec.	min. sec.	min. sec.	min. sec.	min. sec.	min. sec.
Coagulation time (By Wright's method at half blood heat)	4 25 to 4 30	3 15	7 5	6 40	6 55	10 46
Haemoglobin ... (Sahli)	120%	80%	55%	40%	60%	80%

We may not be able to draw any inferences from the smallness of this experiment, but one fact remains that the cerebrospinal fluid from the case of sleeping sickness contained a product which influenced the negative and the positive phase of coagulation, and so far as is known on the subject, it may be the nucleo-proteid.

X. *Sections of the brain tissue.*

The brain tissue was kindly lent to me by Dr. Stephens to whom my thanks are due.

The tissue was embedded in paraffin, sections were cut and stained with haematoxylin and eosin.

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* Allbutt and Rolleston. System of Medicine, Vol. II, Part II, p. 215.

Microscopical appearances.

The perivascular spaces around the blood vessels in the substance of the brain were infiltrated with mononuclear leucocytes, chiefly lymphocytes. This change is typical of the sleeping sickness brain where the infection is due to *T. gambiense.* The infiltration was not enormous. I have applied a method of counting the mononuclears in the perivascular spaces. The sections cut were 8μ in thickness.

Length of the vessel in section in μ	Breadth of the vessel in section in μ	Number of the mononuclears
100	22·8	34
68	68	48
100	68	72
100	100	56
100	100	56
68	68	45
136	68	40
100	85	45
136	100	116
100	85	35
136	100	118
100	85	95
100	85	68
100	85	55
100	85	40
100	85	40
100	85	40
136	100	150
68	68	40
100	34	50
100	34	48
Average 102	72·8	61 mononuclears

That is in a space of 102 μ × 72 μ × 8 μ there are 61 mononuclear leucocytes.

Some of the brain tissue was sent to Dr. Mott, F.R.S., by Professor Ross for his opinion. The report given by Dr. Mott appears below:—

Report on the Examination of Brain Tissue sent by Major R. Ross

Two small portions of brain embedded in paraffin were received; one was a small piece of cerebellum; the other was a small portion of white subcortical tissue, location not definable. Sections of 5μ were cut and stained by polychrome, Giemsa and Heidenhain haematoxylin methods.

All the sections examined showed a well-marked perivascular infiltration with lymphocytes and plasma cells, indistinguishable from that which I have seen in all cases of sleeping sickness. The membranes were absent in both pieces of tissue so I cannot say they were affected. Apparently there was evidence of glia hyperplasia and proliferation in the subcortical tissue. Inasmuch as subcortical and cerebellar tissues always show a more marked perivascular infiltration than the cortex in early cases it would have been more satisfactory if different regions of the cortex could have been examined. For unless the perivascular infiltration affects diffusely the cerebral cortex (seat of consciousness) one would not expect marked lethargy. From an examination of a large number of cases of sleeping sickness I have come to the conclusion that the subcortical structures are affected primarily and that there is a parallelism between the depth of the lethargy and the intensity and diffuseness of affection of the meninges and cortical vessels with neuroglia proliferation and changes in the nerve cells. A large number of the vessels show fibrin filaments indicative of a pneumonic condition.

SOME OBSERVATIONS ON MALARIA IN RELATION TO SPLENIC ENLARGEMENT AND THE TREATMENT OF THE CRESCENTIC STAGE

BY

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During the last two years (1908 and 1909) the city of Bombay was highly infected with malaria, the malignant tertian type being most common. This was most marked in 1908, and large numbers of cases were admitted to hospital. Several of these were in a moribund state, and the blood was found to contain typical rings and crescents in large numbers. In spite of such severe infection, it was noticed that many of them had absolutely *no fever*, and the principal sign of malarial infection, viz., *splenic enlargement, was also absent.*

The patients were in a semi-comatose stupid state, and could not be made to understand questions. In some cases parasites were found only in small numbers, and several slides had to be examined before a ring or a crescent could be detected. The crescents were looked for in several flat thick drops, which were fixed by being heated to about 70° C. for from fifteen to twenty minutes after drying them. This causes the serum to coagulate, and fix the leucocytes, blood platelets, malarial crescents and filarial embryos, if the latter are present. The haemoglobin does not coagulate at this temperature, and can be washed out by dipping the slide in distilled water. Tap water is not suitable if the Giemsa stain is to be used, as the salts in the water precipitate the stain. The slide is then stained with Giemsa's stain in the usual manner. This method is similar to the thick film one devised by Major Ross.

Various drugs were tried to study their effects on the duration of malarial crescents in the blood. It is well known that quinine has not much action on them, and this was seen to be the case in the patients under my care. In the cases under observation, although quinine was always administered in 15 grain doses at the least, there was very little reduction noticed.

Picric acid has long been recommended in malaria, but to my knowledge it is not stated what effects it is supposed to produce on these organisms. In a case of tertian malaria this acid was tried without the least benefit. Its study was taken up in cases of crescents. such of the cases as had the ring forms also present in the blood had the standard treatment with quinine; injections of the latter were also resorted to in severe cases. As control to the picric acid treatment some of the cases were treated only with quinine, either by the mouth alone or with injections as well; whilst others had quinine together with mercury perchloride, arsenic, salol and potassium iodide. The cases treated with picric acid were specially selected for the severity of infection with crescents.

18 cases had picric acid by mouth.
4 cases had injections of picrate of soda (3 c.c. daily of a 4 per cent. solution).
28 cases had quinine (with injections in six cases).
6 cases had quinine together with mercury, arsenic, etc.

The dose of picric acid was gr. 2 twice or thrice a day.

Enormous quantities of quinine (between 500 and 700 grains in different cases, as much as 915 grains having been used in one case) were administered both per os and hypodermically without any marked effect on the crescents, but on the whole under picric acid treatment the crescents seemed to disappear sooner than by any other treatment, as can be clearly seen from Table 'A.' During the time the patient is having the acid, the skin, the nails and the sclerae of the eyes show a yellow tinge as in jaundice, and the urine, too, presents a similar misleading appearance. But on adding an inorganic acid, e.g., hydrochloric or nitric acid, the colour gets paler and not deeper as in the case of urine containing bile. In Table 'A' is shown the number of days during which the crescents were found after quinine treatment alone, as compared with those cases which had quinine and picric acid also, in the cases in which more or less complete observations were possible.

This table clearly shows that cases which received the picric acid treatment lost the crescents earlier than those which had no picric acid. The only case of picric acid treatment where the crescents continued to appear in the blood for sixty-two days was one of the most severe cases of malaria that I have seen. The patient, who was a deaf mute, was quite moribund for days together; while the analogous case without picric acid treatment from the start showed very few crescents—in fact, the ordinary smears did not show any.

The cases which lost their crescents within ten days must be regarded as cases that had probably come at the end of the crescent infection stage and thus lost them early.

Out of four cases in which crescents disappeared between ten and twenty days, three absconded, and hence they are put down as incomplete cases, while it is quite possible that the fourth case had come at the end of the crescentic infection; in fact, he was admitted for croupous pneumonia, and crescents were only found on examination of the blood as a routine method. It is not at all likely that the pneumococcic infection could produce destruction of the crescents, because the case in the simple quinine group that had crescents for sixty-two days was also suffering from the same disease at the time of admission in the hospital. In the quinine group which lost the crescents between twenty and thirty days, one case is marked incomplete for the same reason, viz., that he absconded with the crescents in the blood.

It must be borne in mind that all the cases that received the picric acid treatment were cases of specially severe infection, so that the fact that fourteen out of seventeen lost their crescents between ten and thirty days compares very favourably with the four out of seventeen of the quinine group which lost their crescents during the same period.

Again, on referring to Table 'B,' one finds that the death-rate was higher in the quinine treated cases, whether those had the spleen enlarged or not, as compared with cases which had the picric acid treatment also.

Of course the number of cases is not large, but one must bear in mind that crescent cases as a rule are not found in large numbers, and the two years under consideration were exceptional in the fact that their number was far in excess of those I have seen in past years.

Plehn, at the British Medical Association Meeting in 1908, made the statement that the longest period that the crescents are found in the blood was six weeks; but in two of my cases they were found exactly for two months, and in one of them the last date cannot be determined, as the patient absconded from the hospital. Further, one has to consider the fact that we have no means of determining the date of the first appearance of the crescents in the blood, as all the patients were admitted in a very advanced state of the disease. Thus the total duration of the existence of these parasites must be more than two months.

Another statement by the same author concerns the diagnosis of such cases with crescents by increased urobilin in the urine. The use of an alcoholic solution of zinc acetate and Lugol's solution is described by him as the most delicate test. Both these points I was not able to confirm in my series of cases. Many of these crescents cases showed no increase of urobilin except in the very early stage, and generally at this stage the rings are still present, so that the urobilin is to be attributed rather to the blood destruction by the rings than to the presence of the crescents.

Of the two cases which showed crescents for two months, one had, from the start very few, and still they persisted for the time stated. This patient had only quinine treatment. The other had a very heavy infection, and when admitted was moribund, and so was put on picric acid treatment, but as she hardly ever swallowed her medicine, injections of picrate of soda had to be resorted to for a few days during the second month, and then the crescents soon disappeared.

The injections of picrate of soda were not found to produce any quicker results than the method of administration of picric acid by the mouth; however, in the case mentioned above it was necessary, as the patient would not take the medicines readily.

The method of preparing the solution of picrate of soda for injection is as follows:-

Add 4 grams of acid picric to about 75 c.c. of boiling distilled water, and to this add, drop by drop, a strong solution of sodium hydrate, taking the reaction at the same time, with a solution of di-methyl-amido-azo-benzol till the latter no longer turns red. (Do not stop when the dimethyl turns red and subsequently fades, but go on adding the sodium hydrate solution till no change of colour occurs.) Confirm with phenol-phthalein to see that no change of colour occurs; this latter is necessary to ascertain whether the solution has become too alkaline or not. At this stage, most of the picric acid is dissolved up. Make up the solution to 100 c.c. with distilled water; 3 c.c. of this solution is put up in test-tubes and sterilised at 120° C. in the autoclave. As the water evaporates in these tubes crystals are deposited, which can be readily dissolved either by slightly warming the tube or adding a few drops of sterile distilled water. The injections are no more painful than ordinary injections of vaccine, and do not produce any marked reaction, nor is there any nodule or swelling noticed at the site of injection. Scarcely any pain is present after 24 hours, so that there is no difficulty in injecting about 3 c.c. of this solution on consecutive days.

It will be noticed from the Table 'B' showing the percentages of recovery and death in these cases, that the percentage of death was nearly double in cases not treated with picric acid as compared with those treated with the drug. Again a very interesting point noticed in all these observations was the fact that the mortality was higher in cases where the patient had no obviously enlarged spleen. In some of these cases where a post-mortem examination was obtained, the weight of the spleen was not much above the normal, and in one case it was found quite small and tough.

Cases of benign tertian fever are generally mild, and splenic enlargement is not noticeable in most; however, in my series of twenty-seven cases of a fairly severe type of this infection three cases ended fatally, and none of them showed any splenic enlargement. In the cases of malignant tertian infection, either alone or combined with benign tertian, the mortality was very high in cases where the spleen was not found to be obviously enlarged. Thus the splenic enlargement may be looked upon as a protective response on the part of the organism to the attack of the malarial parasite; and in a case where this response does not occur, it has a tendency to end fatally. Of course in mild cases the response is also slight, but when one considers cases of moderate or extreme severity, this fact is most evident.

This appears to be a natural consequence when one considers the fact that it is the spleen that is most concerned in the destruction of these parasites. The spleen may be called the battlefield and the burial ground of the malarial parasites. Thus the splenic response is, to my mind, a very important feature for purposes of prognosis.

There were some minor points noticed in the course of the investigation which may be mentioned here. Thus one comes across cases where quinine is not absorbed at all per os, and no amount of administration by that method is of any avail. Thus in one of my cases of mixed infection, i.e., benign tertian and malignant tertian. I found that no evidence of an alkaloid could be obtained in the urine by the picric acid and gold chloride tests, although the patient was getting fifteen to thirty grains of quinine hydrochloride daily. Together with this I found that the attacks of fever continued in spite of the quinine by the mouth. Hypodermic injections of quinine had to be resorted to, and the urine at once showed the alkaloidal test. The temperature became normal, and continued to be normal for a few days. Another attack of fever, due probably to a fresh infection, occurred, and quinine was injected with equally good result. However, I had to persist injecting it about half a dozen times and ultimately this had no effect too; the urine at that time was found to be free from quinine, thus showing that the injected quinine was not being absorbed.

The tolerance of the malarial parasite to quinine is also possible, as in some cases the patient gets an attack showing parasites in his blood, although he may be taking ten to fifteen grains of quinine daily. At this stage it is found necessary to increase the amount of the drug.

In an interesting paper on malaria by Darling* it is stated that the number of gametes diminish under quinine administration. He cites only one case, No. 48987 (*vide* Table 'C') to show that no diminution occurred when quinine was withheld. However, one must bear in mind that the patient had non-sexual forms also present in his blood at the time when these observations were made; and as quinine was withheld there is a likelihood of fresh gametes being continually formed.

If one studies the table carefully, one can see that at first there is a diminution in the number of gametes, and only in the last count a sudden increase was noticed. This shows that gametes can disappear even when no quinine is administered, and, therefore, we have up to now no evidence to show that quinine has any effect in diminishing the number of gametes. It is unfortunate that the observation was not made in Case No. 53,937, which was free from young asexual forms. Recently observations on these lines have been undertaken by me which will be published at a future date.

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* Annals of Trop. Medicine and Parasit., Vol. IV, No. 2, 25th July, 1910.

Recently a case of crescents with rings has been put on quinine gr. 30 per day till no rings could be detected. After that the quinine has been stopped, and blood smears and drops have been examined at short intervals to see whether the crescents can diminish without the administration of quinine. The annexed Table 'D' shows that there is a natural tendency for crescents to diminish even when no quinine is administered. The patient has no fever. Liver and spleen are both moderately enlarged. The patient has symptoms of dyspepsia, general debility and irritability of temper. This latter characteristic has been often noticed in crescent cases. The drops show a large and varying number of crescents, although when counted in smears one does not come across such marked differences. This shows that the counting of the smears is not a correct guide for the actual number of crescents in the blood. The diameter of each drop was between 8 and 9 mm., and the crescents were counted in these at an interval of a few days.[*]

TABLE A

No. of days that the crescents took to disappear from the blood when examined in drops	No. of cases treated with picric acid	No. of cases where no picric acid was given	Remarks
Less than 10 days	1	1	
Between 10 and 20 days ...	7	4	[†]3 incomplete
„ 20 „ 30 „ ...	7	4	[†]1 „
„ 30 „ 40 „ ...	1	7	1 died on 34th day
„ 40 „ 50 „ ...	0	0	
„ 50 „ 60 „ ...	0	0	
60 days and over ...	1[‡]	1	

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[*] In Table D it will be noticed that the number of crescents in the drops on the first occasion was very great, and that on the third day there has been a sudden fall, which was kept up to a certain extent in subsequent counts also. Quinine administration was stopped on the 28th September, 1910, and since then none has been given. Is it likely that quinine actually retards the destruction of crescents by lowering phagocytic activity? This is a question worth considering in future observations.

[†] These cases absconded with crescents in their blood, and the results could not be completely ascertained.

[‡] My notes on this case are:—Total quantity of quinine given, 915 grains. Total quantity of picric acid by mouth, 182 grains. Patients hardly ever swallowed these medicines. In spite of the quinine, rings were found in the blood a month after the commencement of treatment, this circumstance being accounted for by the drug not having been taken properly. Injections of picrate of soda were therefore commenced. Ten injections were given, one on every alternate day (in all 30 c.c. of a 4 per cent. solution, i.e., 18 grains of the acid were used during a period of 21 days from 29th December, 1909, to 19th January, 1910). Crescents disappeared in 29 days after the first injection, and they were present altogether for exactly two months.

TABLE B.—Showing the comparative mortality of Malaria cases with and without splenic enlargement, and also of crescent cases under picric acid as compared with ordinary quinine treatment.

Nature of cases	Total cases	Cured	Died	Cured with splenic enlargement	Cured without splenic enlargement	Died with splenic enlargement	Died without splenic enlargement	Remarks
(No Picric) Malignant tertian with rings and crescents .	34	25 (73·53%)	9 (26·47%)	19 (82·61%)*	4 (17·39%)*	2 (22·22%)	7 (77·78%)	* In 2 cases no record has been kept as regards splenic enlargement
Picric Treatment.) Malignant tertian with rings and crescents	22	19 (86·37%)	3 (13·63%)	10 (55·56%)†	8 (44·44%)†	1 (33·33%) †	2 (66·67%)	† Had Picric acid for 2 days only. In 1 case no record of splenic enlargement has been kept.
Malignant tertian with rings only	38	26 (68·40%)	12 (31·60%)	17 (65·40%)	9 (34·60%)	3 (25%)	9 (75%)	
Benign tertian	27	24 (88·89%)	3 (11·11%)	12 (50%)	12 (50%)	0 (0%)	3 (100%)	
Malignant tertian rings plus Benign tertian ...	6	4	2	1	3	1	1	
Quartan	3	2	1	1	1	0	1	

TABLE C.—Case No. 48,987. Darling's Paper

1908	November	November	November	November	December
Date	11	24	27	30	4
Crescents ..	16	27	12	8	20

TABLE D.—Case No. 621. Shewing the natural diminution in the number of crescents without any quinine treatment.

On 21st September, 1910, rings and crescents were detected in smears; on and after 29th September, 1910, when observations were commenced, no rings could be detected.

Smear						Drops		
Date	Poly-morphs	Eosino-philes	Large hyalines	Lym-phocytes	No. of crescents per 100 leuco-cytes	Number of crescents in each drop 8-9 mm. diameter		
1910								
29 September	72	1	5	22	5	149	269	381
30 ,,	69	0	4	27	3	—	—	—
1 October	69	2	3	26	3	18	40	65
2 ,,	70	1	5	24	3	—	—	—
3 ,,	58	0	5	37	3	—	—	—
4 ,,	76	0	7	17	1	—	—	—
5 ,,	79	3	2	26	1	—	—	—
6 ,,	66	2	6	26	0	12	25	20
7 ,,	65	1	4	30	0	—	—	—
9 ,,	67	1	4	18	1	—	—	—
12 ,,	60	3	8	29	1	2	1[illegible]	0
15 ,,	54	11	12	23	0	1	2	0
2[illegible] ,,	62	5	7	26	0	0	0	0

ON THE PECULIAR MORPHOLOGY OF A TRYPANOSOME FROM A CASE OF SLEEPING SICKNESS AND THE POSSIBILITY OF ITS BEING A NEW SPECIES *(T. RHODESIENSE)*[*]

BY

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AND

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PREFATORY NOTE

As already stated in a report to the Advisory Committee for the Tropical Diseases Research Fund, dated May 9, 1910, I noticed early in February, 1910, while examining in class work a stained specimen of rat's blood infected with what was supposed to be *T. gambiense*, a marked peculiarity in the morphology. This peculiarity was so striking that I doubted whether the trypanosome with which I was dealing was really *T. gambiense*. On making enquiries I was told that the strain was derived from a case of sleeping sickness then in Prof. Ross's clinic in the Royal Southern Hospital, Liverpool. To make certain that there was no error in this statement I myself infected a rat from the patient's blood. The same forms were, however, again encountered. After convincing myself that these forms were constantly present in infected rats, and that they were not shown by the rats infected with the old laboratory strain of *T. gambiense* maintained at the Runcorn Laboratory, I decided through pressure of work to ask Dr. Fantham (now working in the Liverpool School of Tropical Medicine, under funds allotted by the Advisory Committee for the Tropical Diseases

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[*] Read before The Royal Society on Nov. 3, 1910, and reprinted from Proc. Roy. Soc., B. Vol. LXXXIII, p. 28, with addenda.

Research Fund) to be so good as to assist me in the description of the morphology of this trypanosome. The following paper is the outcome of our joint work.—[J. W. W. Stephens.]

HISTORY OF THE STRAIN

The trypanosomes used during this investigation were obtained from W.A., male, aged 26, a native of Northumberland, who was infected in North-East Rhodesia in September, 1909. It is necessary to set forth the itinerary of W.A. while in Africa, as he was never actually in an area infested with *Glossina palpalis*, so far as records are available, and indeed was never nearer (Kasama) than some eighty-six miles from such an area.

He first went to South Africa in July, 1904, living in Johannesburg till the end of 1906. He then went to Salisbury for two years. About the end of November, 1908, he left Salisbury for North-Eastern Rhodesia, with a view to prospecting for minerals. The party consisted of two Europeans and ten natives. On the journey northwards he passed through Fort Jameson, Landazi, and Chinsali to Kasama, where he arrived about the beginning of June, 1909. During this northward journey, W.A. passed through an area infested by *Glossina morsitans*. He stayed two months at Kasama, a place from which we have no records of any species of *Glossina*.* On the return journey he passed through Mpika (where *Glossina morsitans* occurs), Serenje (no records of *Glossina*), and Mzaza (where there is *Glossina morsitans*). He left Mzaza on September 10, and travelling along the Luangwa River he reached Feira on September 28. During this part of the journey he would pass through an area infested by *Glossina fusca*, between Mzaza and Hargreaves.

He first fell ill on September 20, but after a rest of two days continued his journey. A short stay was made at Feira, and then the return journey was continued through the Hartley district to Salisbury, where it was found that he and one native† were suffering from trypanosomiasis—parasites being found in his blood in Africa on November 17, 1909.

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* Bagshawe, S.S. Bulletin, No 18. June, 1910, p. 197.

† The infection of the native, at first reported, has since been stated to be a mistake.

The patient stated that he had never been near either Lake Tanganyika, Lake Mweru or the Luapula River, which are known to be infested by *Glossina palpalis*. He thought, himself, that he contracted trypanosomiasis while travelling along the Luangwa River, between Mzaza and Feira. The Luangwa Valley is heavily infested with *Glossina morsitans*, but *Glossina palpalis* has not yet been found along the course of this river.

Dr. Bagshawe's article, already noted, on 'The Transmission in nature of *Trypanosoma gambiense*,' should be consulted as to cases of sleeping sickness contracted in areas infested by *Glossina morsitans*.

We may note that the case of W.A. has been studied by R. Ross and D. Thomson,* who have found a regular periodical increase in the numbers of the trypanosomes in the peripheral blood of the patient from day to day.

It is also of interest to record that the Rhodesian strain of trypanosome from W.A. is somewhat more virulent to rats and guinea-pigs than the old laboratory strain of *T. gambiense*, a fact already confirmed by other workers in the laboratory. Further, this Rhodesian trypanosome is resistant to atoxyl.

MORPHOLOGICAL FEATURES

It may be stated at once that the peculiarity of this Rhodesian trypanosome is that among the stout or stumpy forms some have the *nucleus at the posterior (non-flagellar) end*.

As will be seen from the accompanying figures (Plate XXII, figs. 2-10), the position of this 'posterior' nucleus varies. Starting from the stumpy forms in which the nucleus is in the middle (fig. 1), we have all transitions (figs. 2-8) up to that in which the nucleus is actually terminal (fig. 9) and posterior to the blepharoplast (kinetonucleus).

We may meet here any objections that may be raised that the trypanosomes with posterior nucleus are due to distortion, dried films being used, because:—

1. We have never found them, though persistently looked for, in films from the same animals infected with the old laboratory

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* Roy. Soc. Proc., B., 1910, Vol. LXXXII, pp. 411-415.

strain of *T. gambiense* (figs. 21-25), treated in the same way, *i.e.*, dried films;

2. Further, we have examined the trypanosomes under discussion, by *intra vitam* staining with methylene blue—by this method the posterior position of the nucleus can be seen; and finally,

3. We have fixed wet films with sublimate-alcohol and with osmic vapour respectively, and subsequently stained them with haematoxylin, and found the same forms.

Rats inoculated with the Rhodesian strain usually show a few long thin trypanosomes in the peripheral blood in about three days. The stumpy forms of trypanosomes with the nucleus posterior (figs. 2-10) appear about the fifth or sixth day, and from this time onwards somewhat increase in number up to the seventh to eleventh day. They then form about six per cent. of the trypanosomes present, but may decrease again, varying from day to day.*

These stumpy forms with posterior nucleus (as depicted in figs. 2-8) are 17μ to 21μ long and 2μ to 3μ broad. The nucleus often shows a karyosome, and when at the level of the blepharoplast is often kidney-shaped (fig. 8). There is a well marked blepharoplast (kinetonucleus) and a definite undulating membrane with a flagellar border which terminates in a very short free flagellum. The cytoplasm of these forms is granular, especially at the anterior (flagellar) end, where coarse granules are seen in life, which granules are found to be chromatoid in nature on staining (figs. 2, 3, 5, and 8). These cytoplasmic characters are very like those seen in ordinary stumpy forms of *T. gambiense* (fig. 25). Sometimes a stained line is clearly seen in the stout forms, joining the blepharoplast to the nucleus (fig. 10). We have little evidence as to the relationship of these forms with posterior nucleus to the stumpy forms with the nucleus in the ordinary (central) position (fig. 1), so content ourselves with pointing out that the existence of trypanosomes with posterior nuclei is quite characteristic of this Rhodesian strain, and is so marked a feature that we are always able in films to distinguish it from the old laboratory strain of *T. gambiense*.

These forms with the posterior nuclei are not confined to rats

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* The infected rats live about a fortnight. After the posterior nuclear trypanosomes are once seen they can be found on every subsequent day till the death of the infected host.

30 'passages'), but occur also in *Macacus rhesus* (observed by Dr. Yorke), rabbits, and guinea-pigs infected with this strain. We have been unable to detect these forms in the case of sleeping sickness itself, for the number of trypanosomes present in the blood was always extremely scanty, and in infected rats showing an equally small number of trypanosomes we have also never been able to find them.

A subsidiary but perhaps not unimportant point in the Rhodesian strain is that many of the ordinary long forms have an elongated posterior end, and may be termed 'snout' forms (figs. 13-17). The 'snout' forms occur more especially during the first half or two-thirds of the period of infection in rats. These elongate forms, while not absent in the old laboratory strain of *T. gambiense*, yet are certainly more numerous in the Rhodesian strain, and even by means of this feature we are able to distinguish the strains. 'Snout' forms were frequently seen in the blood of W.A. (figs. 14, 16), and in guinea-pigs. Bruce and Nabarro* have already noted these forms with elongate posterior ends.

Again, although forms with the blepharoplast terminal (figs. 18, 19) are found in the Rhodesian strain, they are less numerous than similar trypanosomes with terminal blepharoplast in the old laboratory strain.

We may also note in passing that pear-shaped (fig. 11) or rounded forms with little or no free flagellum are not uncommon in the Rhodesian strain.

SUMMARY AND CONCLUSIONS

1. We attach some importance to the fact that the patient (now dead) from whom this strain was derived was never, as far as careful enquiries could elicit, in a *Glossina palpalis* area, but had been in many *Glossina morsitans* areas, and very probably in a small *Glossina fusca* area.

2. These posterior nuclear forms (figs. 2-10) have not, as far as we are aware, been described either in the blood of sleeping sickness patients or in the blood of animals infected with *T. gambiense*. Seeing how many competent observers have worked

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* Roy. Soc.: Reports of the Sleeping Sickness Commission, No. I, Pl. 2, Fig. 4.

with *T. gambiense*, this is a striking point and can hardly be an oversight.

3. We ourselves have been unable to find posterior nuclear forms, though constantly searched for, in the blood of rats infected with the old laboratory strain of *T. gambiense*.

4. Posterior nuclear forms exist also, as is well known, in *T. transvaaliense*, and by Lühe these are regarded as developmental stages of *T. theileri*. Whether this be so or not, the condition is not parallel to the one under discussion, for the differences in *T. theileri* are characteristic of all strains of this trypanosome, but the forms which we have described in this paper have, we believe, never been seen before in any strain of *T. gambiense* in man or animals.

5. We are aware that parasitic flagellates may exhibit morphological variations due to changes in metabolism or to differences in environment. However, this Rhodesian strain of trypanosome with posterior nucleus was seen in rats infected from the patient before he was treated with drugs. At first sight also it might appear that the posterior nucleus denoted merely approaching division or even assumption of the round form. However, we have seen division of these posterior nuclear forms (fig. 12). In any case the peculiar morphological feature exhibited by the Rhodesian trypanosome, in the possession of a posterior nucleus, has not been recorded before.

As to the meaning to be attached to these forms, we will consider the following main possibilities:—

(*a*) That we are dealing with a 'variety' or 'local race' of *T. gambiense* due to some change of environment. The possibility in this case exists of *T. gambiense* having been conveyed by a species of *Glossina* other than *Glossina palpalis*, which may account for the peculiar morphology we record; but it must be added that we have no knowledge of any other trypanosome undergoing similar changes owing to such a cause. That it is a 'laboratory' variation we think is out of the question, as all the known laboratory strains of *T. gambiense* are practically identical in morphology.

(*b*) That we are dealing with a new species of trypanosome also producing Sleeping Sickness in man. In support of this view there is firstly, the morphology, which considered alone is strong evidence, since the Rhodesian trypanosome differs from *T. gambiense* more than, for instance, *T. brucei* does from *T. evansi*. Secondly, there is the history, taken in connection with the fact that, so far as we know, *T. gambiense* is conveyed solely by *Glossina palpalis*, but even should it prove that *Glossina palpalis* exists in the districts through which W.A. travelled, then all the more probable is it that we are dealing with a new species of trypanosome.

It seems to us, however, that it is difficult in the absence of further knowledge to discriminate with certainty between these views. Yet, on account of its peculiar morphological features, this Rhodesian trypanosome at least merits a distinct designation as *T. gambiense rhodesiense*, adopting the trinomial nomenclature. Our own view, however, is that we are dealing with a new species of human trypanosome for which we propose the name *Trypanosoma rhodesiense*.

ANIMAL REACTIONS OF *T. RHODESIENSE*

The following table has been compiled from the data obtained by Dr. Yorke. For full details his paper* should be consulted.

Animal	Duration of life in days	
	T. rhodesiense	*T. gambiense*
Monkey—		
M. rhesus ...	9	27
" ...	11	91
" ...	14	—
C. callithricus ...	8	108
" ...	10	149
C. ruber ...	14	—
Rabbit	19	60
"	19	109
"	26	125
"	26	140
"	30	64A
"	32	90A
"	45	150A
"	32A	Neg.
Guinea-pig ...	39	42
" ...	39	120A
" ...	41	134
" ...	52	160
" ...	76	167
" ...	82	—
Dog	9	64
"	12	34A
"	13	—
"	13	—
Goat	45	85
"	46	150A
"	55	—
Donkey	28	—
"	54	—
Pony	38	—

— signifies no experiment. A signifies still alive.

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* Annals Trop. Med. and Parasit., Vol. IV, No. 3, p. 367.

EXPLANATION OF PLATE XXII.

All figures drawn with Abbé camera lucida, using 2 mm. apochromatic objective and 12 compensating ocular (Zeiss). Magnification 1800 diameters approximately.

Figs. 1-20.—Rhodesian strain of human trypanosome. Figures drawn from parasites in the blood of rats, except where otherwise stated.

Fig. 1.—Stout form with nucleus median.

Figs. 2-8.—Stout and stumpy forms, each with posterior nucleus. The nucleus is seen gradually to become more posterior, till it lies behind the blepharoplast (fig. 9).

Fig. 10.—Posterior nuclear form with line connecting blepharoplast and nucleus.

Fig. 11.—Posterior nuclear form becoming rounded.

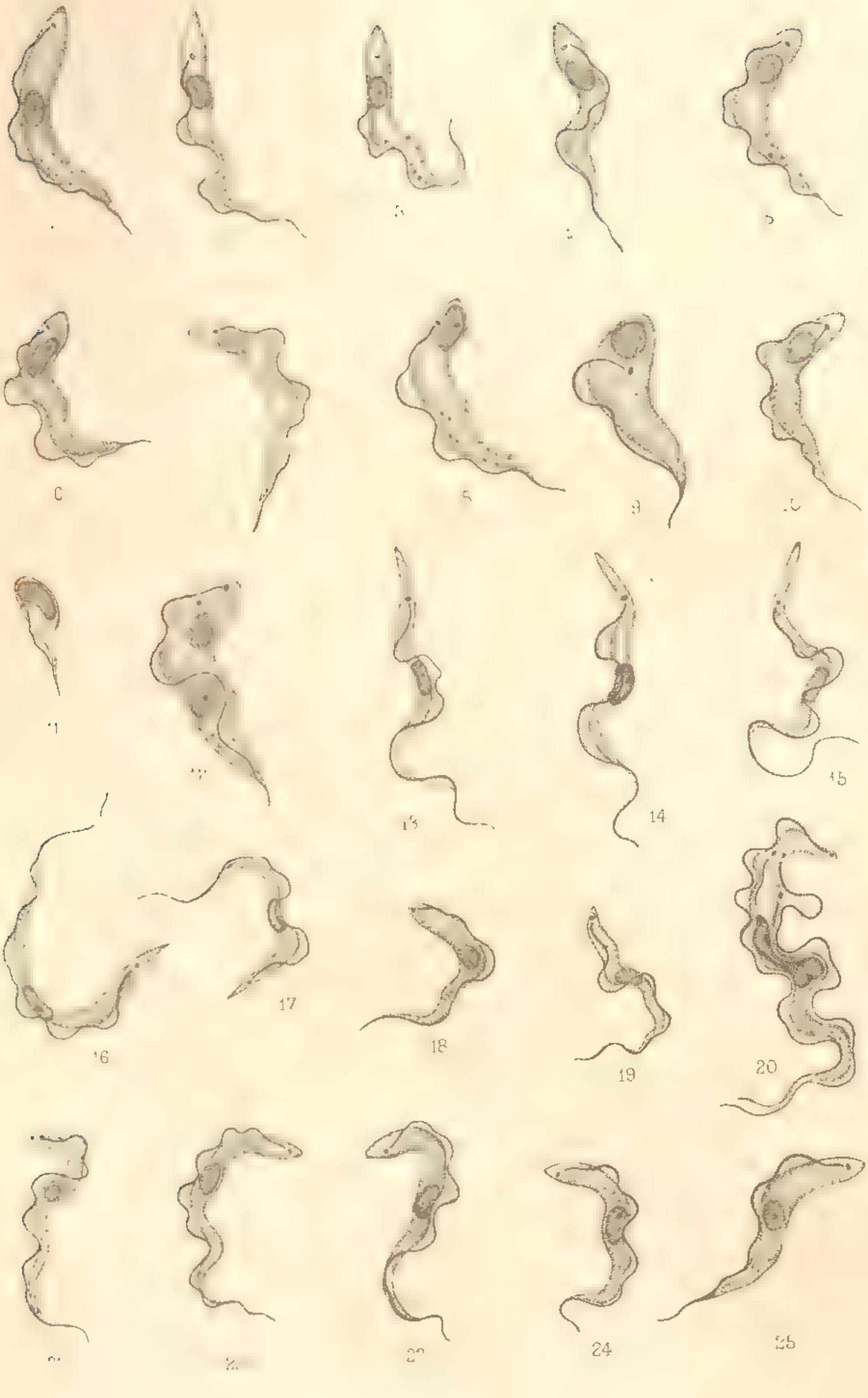
Fig. 12.—Posterior nuclear form showing division.

Figs. 13-17.—'Snout' forms. (Figs. 13, 15 from rat's blood, figs. 14, 16, 17 from man.)

Figs. 18, 19.—Forms with terminal blepharoplast.

Fig. 20.—Multiple division form, with four blepharoplasts and two nuclei. Such parasites are not uncommon in the Rhodesian strain.

Figs. 21-25.—Various trypanosomes drawn from the blood of rats infected with the old laboratory strain of *T. gambiense*



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ON THE PATHOGENICITY OF A TRYPANOSOME (*T. RHODESIENSE*, STEPHENS AND FANTHAM) FROM A CASE OF SLEEPING SICKNESS CONTRACTED IN RHODESIA

BY

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The question as to whether more than one trypanosome acts as a pathogenic agent in man is one which in the past has been much discussed.*

The findings of practically all workers are agreed in deciding that the trypanosomes infecting human beings in different parts of tropical Africa are identical.

Recently, however, doubt has been expressed regarding the identity of a human trypanosome of Rhodesia with that of *T. gambiense*†.

In December, 1909, a patient, W.A. (European), suffering from trypanosomiasis, was admitted into Major Ross's clinic in Liverpool.

So far as could be ascertained the patient had never been into any of the districts in which *G. palpalis* is known to occur. Exactly where the disease was contracted it is impossible to say; possibly, as the patient himself thought, it was in the Luangwa Valley.

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* Castellani. 'Researches on the Etiology of Sleeping Sickness,' Jour. of Trop. Med. and Hyg., p. 167, 1903.

Thomas and Linton. 'A comparison of the animal reactions of the Trypanosomes of Uganda and Congo Free State Sleeping Sickness with those of *T. gambiense*,' Lancet, May 14, 1904.

Plimmer. 'Note on the effect produced on Rats by the trypanosomata of Gambian fever and Sleeping Sickness,' Proc. Roy. Soc., No. 504, p. 388, 1905.

Laveran. 'Sur trois virus de trypanosomiase humaine de provenances differentes,' Comptes Rendus de l'Acad. Sciences, p. 1065, 1906.

Gray and Tulloch. Reports of the S.S. Commission of the Royal Society, No. 8, p. 53.

Bentmann und Günther. 'Beiträge zur Kenntnis des *T. gambiense*,' Archiv für Schiffs- und Tropenhygiene, B. XI, Beiheft 2, 1907.

† Bevan and MacGregor. 'Note on the passage of a Human Trypanosome through domestic animals,' Journal of Comparative Pathology and Therapeutics, p. 160, 1910.

Stephens and Fantham* have described certain morphological peculiarities, which the parasite exhibits in the blood of rats, rabbits and guinea-pigs infected with this strain. As these peculiarities have never been found in *T. gambiense*, the authors consider that this Rhodesian trypanosome is different from *T. gambiense*, and, in fact, a new species, for which they suggest the name *T. rhodesiense*.

In view of these morphological differences and of the fact that the disease was acquired in a district where *G. palpalis* has not yet been found, it appeared desirable to examine the pathogenicity of the parasite for animals of different species, with the object of comparing it with that of our laboratory strain of *T. gambiense* and with the results obtained by other workers with various strains of human trypanosomes.

Recent work has indicated that experiments upon the pathogenicity of a trypanosome must be conducted with certain precautions.

It was found by Thomas and Breinl† that a strain of *T. gambiense*, after passage through a baboon, had greatly increased in virulence. They state that rabbits, rats, cats, guinea-pigs and monkeys inoculated with the strain derived from the baboon developed the disease after short incubation periods, and that death occurred early.

Breinl and Nierenstein‡ found occasional variations in the virulence of *T. gambiense*. Rats infected from a monkey died in three to four days. After passage through guinea-pigs, however, the heightened virulence of the strain was lost and it killed rats again in the normal period, namely, forty to one hundred and twenty days.

Again, it is now generally recognised that long continued passage of a trypanosome through one species of animal tends to increase the virulence of the parasite for this species.

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* 'On the peculiar morphology of a trypanosome from a case of Sleeping Sickness and the possibility of its being a new species (*T. rhodesiense*),' Proc. Roy. Soc., Vol. LXXXIII, p. 28, 1910.

† 'Trypanosomes, Trypanosomiasis and Sleeping Sickness,' Liverpool School of Tropical Medicine, Memoir XVI, 1905.

‡ 'Bio-Chemical and Therapeutical Studies on Trypanosomiasis,' Annals of Trop. Med. and Parasit., Vol. III, p. 395, 1909.

The interesting observation of Schilling and Jaffé[*], that mice, infected with a strain of Ngana, which had been preserved by passage through mice and subsequently treated with arsenophenylglycine, were temporarily immune to the mouse passage strain, but became infected on being inoculated with the same strain which had been kept up in guinea-pigs for two years, shows in what way the parasite can be modified by long passage through distinct animal species.

The work of Uhlenhuth, Hubner and Woithe[†] indicates that the quantity of infective blood injected may play an important rôle in determining the character of the resulting infection.

These authors, working with *T. equiperdum*, found that after inoculation of large quantities of infective blood, infection was delayed, and sometimes did not occur. Similar observations were made by Beck[‡] with *T. gambiense*, who explains the fact on the ground that anti-bodies present in the infective blood act as trypanocides.

The strain which I have used in the following experiments was obtained by inoculating rats with the blood of the patient shortly after his arrival in England, before any medicinal treatment had been administered. The direct strain has been preserved by passage from rat to rat.

Before experiments on animals or other species were commenced, the strain had been passed through a series of twenty rats during a period of about six months.

For our laboratory strain of *T. gambiense* we are indebted to Professor Mesnil, who has kindly informed me that it was obtained by inoculating rats with the cerebrospinal fluid of a case of human trypanosomiasis from the French Congo, in 1905. Since then it has been kept up by passages through rats. Professor Mesnil adds that the strain has gradually become more virulent for rats, which it now kills on an average in twelve days.

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[*] 'Weitere chemotherapeutische Versuche bei Trypanosomenkrankheiten,' Archiv. für Schiffs- und Tropenhygiene, S. 525, 1909.

[†] 'Experimentelle Untersuchungen uber Dourine mit besonderer Berücksichtigung der Atoxylbehandlung,' Arb. a. d. Kaiserl. Gesundheitsamte, B. XXVII, S. 256, 1908.

[‡] 'Experimentelle Beitrage zur Infektion mit *Trypanosoma gambiense* und zur Heilung der menschlichen Trypanosomiasis,' Arb. a. d. Kaiserl. Gesundheitsamte, S. 318, 1910.

In my experiments, the different animals were always inoculated with the blood of infected rats, so as to avoid any alteration in virulence comparable to that described by Thomas and Breinl, resulting from passage through a baboon.

With a view to avoiding any discrepancy arising from injection of widely varying numbers of trypanosomes, the injected blood was diluted with citrated saline solution, until it contained a definite number of parasites per cubic millimetre.

This was accomplished by diluting the blood of a heavily infected rat with many times its volume of citrated salt solution and then counting the trypanosomes in the diluted solution by means of the Thoma-Zeiss haemocytometer. The mixture was then further diluted with normal saline until one cubic millimetre contained 500, 1,000, 5,000 or 10,000 trypanosomes.

When cover-slip preparations of such solutions containing 1,000 trypanosomes per c.mm. were examined microscopically, it was found that there was approximately one parasite to a field (Zeiss objective DD; eyepiece No. 4).

MONKEYS.

Rhodesian strain. Six experiments. The following species were used, *Macacus rhesus*, *Cercopithecus callitrichus* and *Cercopithecus ruber*. In all, the disease ran a very rapid course. Trypanosomes appeared in the blood after short incubation periods, and were subsequently always present—often in very large numbers—until death occurred. Posterior nuclear forms* of the parasite were found in every case except Monkey 6 (*Cercopithecus ruber*), where the parasites, although constantly present, were never numerous, the largest number found being ten to a field. With the exception of emaciation the animals presented no symptoms until within two or three days of death, when they lay at the bottom of their cages in a drowsy condition, ate little, if anything, and hardly responded to stimulation. The temperature never rose above

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* Forms in which the nucleus was either close to the blepharoplast or actually posterior to it.

104° F. in any of the animals, but usually fell considerably shortly before death. Incubation period, three to five days; average four days. Duration, eight to fourteen days; average eleven days.

TABLE 1.—Monkeys infected with the *Rhodesian Strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. *Macacus rhesus.* Weight 2210 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	5th day	14th day	Parasites always present in large numbers. Many posterior nuclear forms found. Weight at time of death, 1850 g.
2. *M. rhesus.* Weight, 1670 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	11th ,,	Parasites numerous on the sixth and seventh days. Posterior nuclear forms present. Subsequently the number of trypanosomes diminished. Weight at time of death, 1500 g.
3. *M. rhesus.* Weight, 5370 g.	Subcutaneously. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th ,,	9th ,,	Parasites present in large numbers. Posterior nuclear forms found. Weight at time of death, 5000 g.
4. *Cercopithecus callitrichus* Weight, 2520 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	4th ,,	10th ,,	Parasites present in considerable numbers from the sixth day until death. A few posterior nuclear forms seen. Weight at time of death, 2100 g.
5. *C. callitrichus.* Weight, 3160 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	4th ,,	8th ,,	Parasites numerous. Many posterior nuclear forms found. Weight at time of death, 2550 g.
6. *C. ruber.* Weight, 4150 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th ,,	14th ,,	Parasites fairly numerous (10 to a field) on the ninth day; otherwise scanty. No posterior nuclear forms found. Weight at time of death, 3600 g.

T. gambiense. Four experiments. *Macacus rhesus* and *Cercopithecus callitrichus* were used. With this strain the disease ran a much more chronic course. Parasites were usually scanty, or absent from the peripheral circulation. Incubation period, three t twenty-one days; average twelve days. Duration, twenty-seven days to over four months.

Table 2.—Monkeys infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. *Macacus rhesus.* Weight, 2490 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	3rd day	27th day	Parasites always scanty or absent. Weight at time of death. 2130 g.
2. *M. rhesus.* Weight, 3380 g.	,, ,,	5th ,,	91st ,,	Parasites scanty or absent generally, but fairly numerous during the last few days. Weight at time of death, 2600 g.
3. *Cercopithecus callitrichus* Weight, 2450 g.	,, ,,	21st ,,	149th ,,	Parasites as a rule scanty or absent. Animal very drowsy during last week. Weight at time of death, 1820 g.
4. *C. callitrichus.* Weight, 2730 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	6th ,,	108th ,,	Parasites occasionally present in large numbers, but usually scanty or absent. Weight at time of death, 1750 g.

RABBITS.

Rhodesian strain. Eight experiments. In these animals the disease was fairly acute. Incubation period, from three to fourteen days; average eight days. Duration, nineteen to forty-five days; average twenty-eight days. Parasites were almost constantly present in the blood, although usually only in small numbers, except during the last few days, when they were generally present in considerable numbers. Posterior nuclear forms were usually to be found in small numbers on the occasions when the blood contained numerous parasites.

The symptoms presented were those usually found in rabbits suffering from trypanosomiasis, viz, blepharo-conjunctivitis, oedema of the head and ears, purulent discharge from the nose and eyes, loss of hair, emaciation and anaemia. Probably, owing to the shorter course of the disease, these symptoms were not so marked as in the rabbits infected with *T. gambiense*, where the disease is much more chronic. Two of the animals were very drowsy for a week before death (Nos. 5 and 6).

TABLE 3.—Rabbits infected with the *Rhodesian strain*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	9th day	45th day	Parasites scanty until the last few days, when they were fairly numerous, and when a few posterior nuclear forms were seen.
2. Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	32nd ,,	,, ,,
3. Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	7th ,,	26th ,,	,, ,,
4. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	5th ,,	26th ,,	,, ,,
5. ,, ,,	9th ,,	19th ,,	Parasites scanty, but constantly present until time of death, when they were fairly numerous (10 to a field). No posterior nuclear forms found. The animal was very lethargic during the last week.
6. ,, ,,	9th ,,	19th ,,	,, ,,
7. Intraperitoneally. 1 c.c. of diluted blood solution containing 250 trypanosomes per c.mm.	14th ,,	—	Animal alive after 32 days. Parasites constantly present, but scanty. No posterior nuclear forms seen. Marked oedematous swelling of skin of head, loss of hair, ears thickened.
8. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	8th ,,	30th ,,	Parasites only found in small numbers until last few days, when they were fairly plentiful (5 to a field). A few posterior nuclear forms found.

T. gambiense. Eight experiments. The disease was chronic. Parasites were only occasionally found in the peripheral blood, and always in very small numbers. Incubation, five to nineteen days, average twelve days. In one animal parasites were never found in the blood during the whole course of the disease. It presented the usual symptoms of infection, viz., oedema, loss of hair, muco-purulent discharge from nose and eyes, etc. Duration, sixty days to more than five months.

TABLE 4.—Rabbits infected with *T. gambiense*

	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1.	**Intraperitoneally.** 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	5th day	125th day	Parasites only occasionally found, and always scanty.
2.	**Intraperitoneally.** 2 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	—	60th ,,	Parasites never found in blood. It presented the usual signs of disease.
3.	**Intraperitoneally.** 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	9th ,,	140th ,,	Parasites rarely found, and only in very small numbers.
4.	,, ,,	15th ,,	109th ,,	,, ,,
5.	**Intraperitoneally.** 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th ,,	—	Animal alive after five months. It presents marked symptoms of disease, e.g., loss of hair, etc. Parasites rarely present in blood.
6.	**Intraperitoneally.** 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	19th ,,	—	Animal alive after three months. Parasites occasionally found in small numbers.
7.	,, ,,	15th ,,	—	Animal alive after 64 days. Parasites rarely found, and very scanty.
8.	,, ,,	—	-	Parasites have not yet been found in blood. Animal perfectly normal after 23 days.

GUINEA-PIGS.

Rhodesian strain. Six experiments. Parasites generally present in the blood, often in considerable numbers. Posterior nuclear forms were usually found when the parasites were numerous. The animals presented no symptoms, and the disease ran a fairly chronic course. Incubation period, three to fifteen days; average eight days. Duration, thirty-nine to eighty-two days; average fifty-five.

TABLE 5.—Guinea-pigs infected with the *Rhodesian strain*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	15th day	41st day	Parasites scanty except during the last week, when they were fairly numerous (15 to 20 to a field). Posterior nuclear forms found.
2. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	52nd ,,	,, ,,
3 ,, ,,	12th ,,	39th ,,	Parasites present in blood regularly, but in small numbers. No posterior nuclear forms seen.
4 ,, ,,	7th ,,	39th ,,	Parasites constantly present, generally in large numbers. Many posterior nuclear forms found.
5. Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm	6th ,,	82nd ,,	,, ,,
6. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	76th ,,	Parasites always scanty until the last few days, when they were fairly numerous (10 to a field). Posterior nuclear forms seen.

T. gambiense. Five experiments. Parasites usually present only in very small numbers or absent. Disease very chronic. Incubation period, three to forty days; average fifteen days. Duration, forty-two days to five and a half months; average more than four months.

Table 6.—Guinea-pigs infected with *T. gambiense*

	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1.	Intraperitoneally. 1 c.c. of diluted blood solution containing (?) trypanosomes per c.mm.	40th day	134th day	Parasites not found in blood (except on 40th day) until the last two days, when they were present in small numbers.
2.	„ „	3rd „	167th „	Parasites rarely found in small numbers.
3.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th „	42nd „	Parasites regularly present, and sometimes in considerable numbers (20 to 30 to a field).
4.	„ „	13th „	—	Parasites only found on three occasions in very small numbers. Animal alive and well after four months.
5.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	12th „	160th „	Parasites rarely found in very small numbers.

DOGS.

Rhodesian strain. Four experiments. The trypanosome proved to be extremely virulent in dogs. In Dogs 1 and 2, parasites were present in the blood in large numbers, and many posterior nuclear forms were seen. Trypanosomes, although constantly present, were never very numerous in Dogs 3 and 4, and none of the above varieties were observed. The animals rapidly lost their weight. Incubation, four to five days; average four days. Duration, nine to thirteen days; average twelve days.

TABLE 7.—Dogs infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Mongrel. Weight, 5100 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	4th day	9th day	Parasites present in large numbers. Posterior nuclear varieties seen. Weight at time of death, 4300 g.
2. Irish terrier. Weight, 12,050 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 250 trypanosomes per c.mm.	5th ,,	13th ,,	Parasites present in large numbers. Posterior nuclear forms seen. Weight at time of death, 10,460 g.
3. Collie Weight, 7950 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th ,,	13th ,,	Parasites 10 to a field on the sixth to ninth days. Subsequently they were scanty. No posterior nuclear forms found. Weight at time of death, 7150 g.
4. Spaniel. Weight, 17,090 g.	,, ,,	4th ,,	12th ,,	Parasites scanty. No posterior nuclear forms seen. Weight at time of death, 15,020 g.

T. gambiense. Two experiments. In both animals the disease ran a chronic course. Parasites were, as a rule, only found in small numbers. Incubation period, twelve to sixteen days; average fourteen.

TABLE 8.—Dogs infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Irish terrier. Weight, 9500 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	12th day	64th day	Parasites found fairly regularly in small numbers. On the last two days trypanosomes were present in large numbers (30 to a field).
2. Mongrel. Weight, 10,250 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	16th ,,	-	Parasites found fairly constantly, but usually in very small numbers. Animal still alive on 34th day.

GOATS.

Rhodesian strain. Three experiments. In these animals the disease ran a fairly rapid course. Parasites were not infrequently seen in the blood, but in very small numbers only, and the posterior nuclear variety was never found. The only symptoms observed were: loss of weight, oedematous swellings—accompanied by slight loss of hair—of the skin and subcutaneous tissues over the nasal bones and beneath the eyes, and interstitial keratitis. The temperature of Goat 1 rose to 107° F. on the seventh day after inoculation, but quickly returned to normal (103-4° F.). The other two animals never exhibited any rise of temperature. Incubation period, four to eleven days; average seven days. Duration, forty-five to fifty-five days; average eight days.

TABLE 9.—Goats infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Medium ♀	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	6th day	55th day	Parasites scanty. No posterior nuclear forms seen.
2. Large ♀	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	4th ,,	46th ,,	Parasites scanty. No posterior nuclear forms seen.
3. Large ♂	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	11th ,,	45th ,,	Parasites scanty and rarely found. No posterior nuclear forms seen.

T. gambiense. Two experiments. Both animals became infected, but the disease was exceedingly chronic. Parasites have never been found in the blood of Goat 1, but two rats, inoculated with its blood on the forty-fourth day, became infected after an incubation period of twelve days. The animal is still alive, more than five months after inoculation, and has presented no symptoms of disease. Rats inoculated with its blood, on the hundred and fifty-second day, did not become infected. Parasites (1 to 15 fields) were found in the blood of Goat 2 on the nineteenth day after inoculation, but were never observed subsequently. On the seventy-second day slight swelling of the face was noticed. Beyond slight wasting no other symptom was exhibited. Death occurred on the eighty-fifth day.

TABLE 10.—Goats infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Large ♀	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	–		Parasites never found in blood. Rats inoculated with its blood on the 44th day became infected, but not those inoculated on the 152nd day. Animal alive and well after more than five months.
2 Medium ♀	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	19th day	85th day	Parasites only found once (on the 19th day) in very small numbers. Rats inoculated with its blood on the day of death became infected.

DONKEY AND HORSE.

Rhodesian strain. Two donkeys and a horse were easily infected.

Donkey 1. Inoculated subcutaneously with a small amount of the virus. Parasites first appeared in the circulation on the fifth day. From the sixth to the eighth day trypanosomes were present in fair numbers (3 to 10 to a field), and a few posterior nuclear forms were found. Subsequently parasites were constantly present in very small numbers until the last week when the number again increased, and the posterior nuclear variety were again found. Temperature remained constant (103-4° F.) throughout the disease. Beyond wasting and an oedematous condition of the skin and subcutaneous tissues of the face, there were no symptoms, until the forty-seventh day, when it was noticed that the animal was very weak. The next day it was down and unable to rise. Death occurred on the fifty-fourth day.

TABLE 11.—Donkeys and Horse infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Donkey ♀ Weight, 364 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	5th day	54th day	Parasites constantly present, but, as a rule, scanty, except on last three days, when they were fairly numerous (10 to 20 to a field). Posterior nuclear forms found. Weight a week before death, 290 lbs.
2. Donkey ♂ Weight, 324 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	7th ,,	28th ,,	Parasites constantly present in small numbers. No posterior nuclear forms found. Weight five days before death, 280 lbs.
3. Pony ♂ Weight, 644 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th ,,	38th ,,	Parasites constantly present, but only in small numbers until within a few days of death, when 3 to 5 to a field were found. A few posterior nuclear forms were seen. Weight three days before death, 548 lbs.

Donkey 2. Inoculated subcutaneously. Incubation period seven days. Parasites were subsequently constantly present in the blood in small numbers. No posterior nuclear forms were seen. Temperature 103-104° F. The only signs of disease were progressive wasting and debility. Death took place on the twenty-eighth day.

A *Horse* inoculated subcutaneously with a small dose of the Rhodesian strain exhibited parasites in its blood on the seventh day. Trypanosomes were subsequently regularly found, there being sometimes as many as five to a field, but, as a rule, they were scanty. A few posterior nuclear forms were found on the thirty-second day, at a time when the total number of parasites in the blood was about five to a field. The animal rapidly lost weight. Before infection it weighed 650 lbs., five days before death 548 lbs. The temperature remained constant, between 103° and 103·8° F., until a few hours before death, when it fell to 99° F. On the twenty-fourth day after inoculation the animal appeared ill for the first time. A few days later distinct swelling of the skin, subcutaneous tissues below the eyes and over the nasal bones was noticed. This condition was accompanied by some slight loss of hair and persisted until death. On the thirty-fifth day the animal was very ill. It had lost nearly 100 lbs. in weight, could hardly stand and refused food. The cornea of the left eye was hazy, and the conjunctiva slightly injected. The next day the animal was down and could not get up. Death occurred on the thirty-eighth day. At the time of death parasites were present in the blood in fair numbers (4-5 to a field).

MICE.

Rhodesian strain. Twenty experiments. The disease ran an acute course. The animals were inoculated intraperitoneally with $\frac{1}{4}$ c.c. of blood containing approximately 500 trypanosomes to a cubic millimetre. Parasites were usually present in fair numbers, and the posterior nuclear forms frequently found. Incubation period, two to five days; average three days. Duration, three to twenty days; average eleven days.

RATS.

Rhodesian strain. Forty-five experiments. The strain has been preserved by passage through these animals. In each case the rats were inoculated intraperitoneally with 1 c.c. of infective blood well diluted with citrated saline solution—a cover-slip preparation of which showed, as a rule, one to five trypanosomes to a field. The disease was fairly acute, and parasites were often present in the blood in considerable numbers. Posterior nuclear forms were usually found when the blood contained numerous parasites. Very little alteration in virulence has been observed after a year's passage through these animals. The two rats infected directly from the patient died in six and twelve days, respectively. The average duration of the disease in the first ten passages was 17·5 days, and in the last ten, 20 days. Incubation period, one to seven days, average four days. Duration, six to forty-five days; average nineteen days.

T. gambiense. Fifty-four experiments. Incubation period, two to ten days; average four days. Duration, six to twenty-nine days average fourteen days.

With a view to comparing the animal reactions of the *Rhodesian Trypanosome* with those of *T. gambiense*, the above results, and those obtained by other workers* with various strains of human trypanosomes, are summarised in Table 12.

It is evident that the Rhodesian trypanosome differs very markedly from *T. gambiense* in its pathogenicity for various species of animals. In monkeys (*Macacus rhesus*, *Cercopithecus callitrichus* and *Cercopithecus ruber*), dogs, rats and mice the disease runs a very acute course. Large numbers of trypanosomes were usually present in the blood. Rabbits, guinea-pigs and goats were easily infected, and died in a much shorter time than animals of the same species infected with *T. gambiense*, and parasites were much more constantly found in the blood and in greater numbers. Two donkeys and a horse quickly succumbed to the disease, trypanosomes being regularly observed in the peripheral circulation.

These results show that in so far as virulence is concerned the parasite is widely different from *T. gambiense*—in fact it more closely resembles *T. brucei* in this respect than *T. gambiense*—and support the view advanced by Stephens and Fantham, on morphological grounds, that we have in '*T. rhodesiense*' a new species.

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* Reports of the Sleeping Sickness Commission of the Royal Society.

Thomas and Linton. *Loc. cit.*

Laveran. *Loc. cit.*

Brumpt et Wurtz. 'Maladie du Sommeil expérimentale chez les Souris, Rats, Cobayes, &c., Comptes Rendus Soc. Biologie, T. LVI, p. 567, 1904.

Beck. 'Experimentelle Beiträge zur Infektion mit *Trypanosoma gambiense*,' Arbeiten a. d. Kaiserl. Gesundheitsamte, S. 318, 1910.

Bentmann und Günther. *Loc. cit.*

TABLE 12.—Summary of the results obtained in various animals with the *Rhodesian strain* and *T. gambiense* respectively, and those of other workers with various strains of *T. gambiense*

	Rhodesian strain		*T. gambiense*		RESULTS OBTAINED BY VARIOUS WORKERS WITH DIFFERENT STRAINS OF HUMAN TRYPANOSOMES											
					Royal Society Commissioners in Uganda		Thomas and Linton		Laveran		Brumpt and Wurtz		Beck		Bentmann and Gunther	
	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration
Rat ...	4 days	19 days	4 days	14 days	20 days	3-8 + mths.	8 days	45-388 days	G.* 13 days U. 11 ,, K. —	62 days 77 ,, 81 ,,	3-4 days	4-5 months	3-5 days	3-4 mths.	U. 4·9 days K. 12·5 ,,	69 days 81 ,,
Mouse ...	3 ,,	11 ,,	—	—	—	—	4-7 days	11-14 days	G. 13 ,, U. 8-20 ,, K. 30-45 ,,	57 ,, 153-216 ,, 281-351 ,,	2-3 ,,	3-4 ,,	2-3 ,,	8-10 days	U. 5·3 ,, K. 4·9 ,,	49 ,, 90 ,,
Guinea-pig ...	8 ,,	55 ,,	15 ,,	3-5 + mths.	—	—	12-45 ,,	3-8 mths.	G. 26 ,, U. 16 ,, K. 20 ,,	100 ,, 81 ,, 100 ,,	Always negative	1½ ,,	2-3 ,,	3-5 mths.	U. six experiments with no positive result K. only 2 positive results out of 21 experiments	
Rabbit ...	8 ,,	28 ,,	12 ,,	3-5 + mths.	—		5-15 ,,	54-128 days	—	—	Always negative	1-4 + ,,	8-14 ,,	4-5 mths.	U. 9 ,, K. 9 ,,	28·6 days 49 ,,
Dog ...	4 ,,	12 ,,	14 ,,	More than 1½ months	22 ,,	Some months	5-10 ,,	33-43 ,,	—	—	17 days	66 days	2-3 ,,	1½-4 mths.		
Monkey—																
Macacus rhesus	4 ,,	11 ,,	4 ,,	1-3 mths.	9-22 days	2-8 mths.	—	—	—	—	8-9 ,,	16 days to 2 months	—	—		
Cercopithecus callitrichus	4 ,,	9 ,,	13 ,,	3-5 ,,	9-40 ,,	2½-12 ,,	—		—	—	7 ,,	19-88 + days	—	—	U. 10 ,, K. 12 ,,	82 ,, 105 ,,
Cercopithecus ruber	4 ,,	14 ,,			—	—		—	—	—	7 ,,	24 days	—	—		
Goat ...	7 ,,	48 ,,	Parasites only found once on 19th day in Goat 1; never found in Goat 2	3-5 + mths.	Parasites found on 13th day after 5th inoculation in one goat. Other 2 goats negative	One animal died on 22nd day. Other two recovered	-	All died: duration not stated	—	—		—	—	—		
Donkey ...	6	41 ,,	—	—	3 were inoculated, but did not become infected	—	Parasites found on rare occasions	—								
Horse ...	7 ,,	38 ,,	—				30 days	Recovered						—		

* G. signifies *T. gambiense*, from Gambia; U. Trypanosome of Sleeping Sickness in Uganda; K. Trypanosome of Sleeping Sickness in Congo.

My observations as to the morphology of the parasite confirm those of Stephens and Fantham regarding the existence of posterior nuclear forms in infected rats, guinea-pigs and rabbits. I have also found them in monkeys (*Macacus rhesus*, *Cercopithecus callitrichus*), dogs, mice, horse and donkey.

I have not, however, succeeded in finding these forms in the blood of the patient himself, in spite of very careful daily examinations during a period of over three months.

Why the posterior nuclear forms should be apparently absent from the patient's blood is not very obvious. Trypanosomes were always scanty and possibly these forms may have been missed, although nearly two thousand parasites were examined without meeting a single example of the form in question. However, the posterior nuclear variety is not usually seen in infected animals until the blood is fairly heavily infected, when they may form as many as five or six per cent. of the total parasites present.

Sections of the nervous system of the patient exhibit well-marked perivascular infiltration and leptomeningitis. The spleen was greatly increased in size and deeply pigmented (malarial ?). The cervical, axillary, mesenteric and inguinal lymphatic glands were considerably enlarged, and presented the usual histological appearances.

ON THREE NEW SPECIES OF THE GENUS GLOSSINA, TOGETHER WITH A DESCRIPTION OF THE HITHERTO UNKNOWN MALE OF *GLOSSINA GROSSA*, BIGOT

BY

ROBERT NEWSTEAD, M.Sc., A.L.S., &c.

(*Received for publication* 15 *December*, 1910)

The discovery of the three species of *Glossina* herein described was brought about by an examination of the morphological characters of the genital armature of the males. In the first instance some doubt had been entertained regarding the correct specific identity of certain dark forms of tsetse flies which had been placed in the collections of this School as doubtful examples of *Glossina morsitans*, West. The examples in question were captured by Major Dansey-Browning, in the Bahr-el-Ghazal Province of the Sudan, during the winters of the years 1905 and 1906. These captures were recorded* by him in the year 1908, with a note as to the peculiar dark colour which they presented in life. More recently a long series of tsetse flies, which were collected by Dr. F. W. McCay, on the Katangum River, between Katangum and Geidam, Northern Nigeria, were presented to this School. These examples were found to be specifically identical with those taken in the Sudan. On comparing the genital armature of these dark forms with those of typical *G. morsitans* from Rhodesia, it was found that they were structurally distinct; it became necessary, therefore, to raise the dark forms to specific rank. On the examination of some *western* examples of the so-called *Glossina morsitans* one found also that these presented the same morphological characters as those of the dark forms both from the Sudan and Katangum. My interpretation of these specific differences is that all the tsetse flies occurring in the western regions of Africa which have hitherto been considered as *Glossina morsitans* will prove, on further examination, to be referable to the species herein described as *Glossina submorsitans*, n. sp.; what the exact distribution of this insect is remains to be seen, as at the present moment I have no further material available for examination.

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* Jour. Roy. Army Med. Cor., Vol. X, p. 427.

The examination of the morphological characters of the armature of the males of all the other known species of the genus *Glossina* has revealed the fact that there are four distinct species of that group of tsetse flies, of which *G. fusca*, Walk., may be taken as a type. These are *G. longipennis*, Corti, and *G. brevipalpis*, Newstead, from the eastern side of the continent; while *G. fusca*, and *G. grossa*, Bigot, are, as far as I can gather at present, the western representatives of this group.

With regard to the large tsetse fly herein referred to as Bigot's *Glossina grossa*, I had at first thought this to be a new and hitherto undescribed species; but, on further consideration, I came to the conclusion that, as it agreed so well with the author's description,* it must be referable to this insect; all the more so on account of the statement made by Austen,† that 'the apex of the third joint of the antennae is very prominent.' Austen, who had evidently examined Bigot's type, did not, however, consider this marked character of specific importance, and sunk *Glossina grossa* as a synonym of *G. fusca*, Walk. Should my examples eventually prove to be specifically distinct from *G. grossa* I would suggest the specific name *nigrofusca*.

It may be interesting to add also, in this connection, that there are no structural differences between *Glossina palpalis* and the var. *wellmanni*; so that for strictly medical purposes I would suggest that the var. *wellmanni* should be sunk, all the more so seeing that *Glossina palpalis* is given to great variation in size, and that there are colour varieties or forms which are intermediate between typical *palpalis* and its var. *wellmanni*.

I have, so far, been able to examine but one specimen of *Glossina pallidipes*, Aust. This proves to be specifically identical with *Glossina longipalpis*, Wied. The most, therefore, that one can do in the light of this discovery is, I feel, to make *G. pallidipes* a colour variety of *G. longipalpis*.

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* Ann. Soc. Ent. Fr., Vol. LX, p. 377 (1901). Female only.

† Monograph of Tsetse Flies, p. 97.

A more extensive paper dealing with the morphological characters of the genital armature of all the known tsetse flies is in the course of preparation, and will be published early in the new year, together with a series of illustrations which will aid the student in the exact determination of this genus of pathogenic insects.

Glossina submorsitans, n. sp.

Nearly related to *Glossina morsitans*, Westw., in having the tips of all the tarsi dark. There are two well-marked varieties: a *dark form* and a *light form*. The former may be readily distinguished by its general dusky or dull vinous-grey colour and generally smaller size; the pale form, however, very closely resembles *G. morsitans* in the general colour of the abdomen; but the bilateral abdominal bands are:—

(*a*) much more clearly and sharply defined.

(*b*) equally and more narrowly interrupted in the median line, between the *third*, fourth, and fifth segments.

(*c*) slightly rounded medially and suddenly tapering towards the lateral margins.

In *G. morsitans* the bands are:—

(*a*) not so sharply defined medially.

(*b*) gradually interrupted in the middle line, the space between the *third* being much greater than the space between the fifth, and the dark colour gradually shades off into the pale colour forming the median line.

(*c*) *broadly* rounded medially, and a very gradually tapering towards the lateral margins.

The morphological character of the genital armature of the males of these species, respectively, are easily separable by the form of the median processes.

DARK FORM.—*Thorax* grey, with the usual dark brownish longitudinal markings, generally relatively small, sometimes reduced to elongated spots. *Abdomen* dull olivaceous grey, with a faint vinous tinge, most pronounced on the second and third segments; second segment with a large bilateral infuscated blotch, often enclosing a small but distinct sub-central blackish spot, but occasionally also the spots are separated, and in some examples the small dark one only remains; the third to the sixth segment, inclusive, with sharply defined and conspicuous blackish bands, narrowly interrupted in the median line. *Legs* with the tips of the last two segments of the front and middle tarsi black; the last two segments of the hind tarsi uniformly black.

Length.—7 to 8 mm.; length of wing, 7 to 8 mm.

Type, male and female (Coll. British Museum), Katangum River, between Katangum and Geidam, Northern Nigeria. Dr. F. W. McKay.

PALE FORM.—*Thoracic* markings similar to those in the dark form; but the pleurae are generally paler. *Abdomen* greyish buff, with a pale vinous tinge; bilateral blackish bands, very sharply and clearly defined and narrowly interrupted in the median line; those on the *third, fourth, and fifth segments* being almost equally separated by the pale median stripe; the bands are also only very slightly rounded medially, and rather suddenly so towards the lateral margins. *Legs* as in the dark forms.

Two co-type males and a female (Coll. Brit. Museum) were captured by Dr. A. Kinghorn at Sabiya-Boge, Northern Ashanti, 24th April, 1910. Eight additional specimens were also captured by Dr. Kinghorn in various localities in Ashanti, all of which have pale abdomens as in the types.

Glossina brevipalpis, n. sp.

This tsetse fly may be easily recognised from the two western representatives of the fusca group (*G. fusca* and *G. grossa*) by its shorter and stouter palpi, its generally paler colour, and somewhat indefinite thoracic markings; in the morphological characters of the male armature; also by the great length of the median process; the presence of a long bilateral row or band, of squamose spines; and in the form of the harpes.

Length.—10-12 mm.; length of wing, 11-11·50 mm.

MALE.—*Head* with the front uniformly pale ochreous brown, frontal margins brighter. *Antennae* pale ochreous, with the anterior half infuscated; arista infuscated at the base. *Eyes* slightly converging. *Palpi relatively short and stout. Thorax* dark brown, with the markings dusky grey-brown, these vary in intensity, but are usually more or less indefinite.

Abdomen dark brown; second segment scarcely paler than the others; margins and posterior angles sometimes narrowly paler.

Legs pale ochreous brown; last two segments of middle and hind tarsi dark, blackish; last two segments of front tarsi either with or without brownish tips; front femora infuscated dorsally, and slightly so laterally on the inside; hind tibiae with a more or less distinct infuscated band; venter, in examples which have not partaken of a meal of blood, pale ochreous brown, margins of the third to the sixth infuscated; stigmata dusky ochreous.

FEMALE.—Colour pattern closely resembling that of the male, but with the hind and lateral margins generally more strongly pronounced.

Type, female, Songwe River, N. Nyasa, 7th January, 1909 (Dr. J. B. Davey). Co-type, male, near Kporo, N. Nyasa, 5th August, 1909 (Dr. J. B. Davey).

Glossina grossa, Bigot.

Ann. Soc. Ent. Fr., Vol. LX, p. 377 (1891). Female only.

This species is distinguishable from *Glossina fusca* by its generally darker colour; the darker hind tarsi in the male; the narrow lines on the thorax and the strongly pubescent character of the antennae. Morphologically also in the male armature by the form of the clasperettes, the large group of squamiform spines over the harpes, and the broad dark sclerite arising from its anterior portion near the base of the mantle.

Length.—11-12 mm.; length of wing, 11-11·50 mm.

Thorax very dark brown to blackish brown, with *very narrow pale longitudinal stripes, scarcely broader than the pale transverse suture. Abdomen* blackish brown, with the second (largest) segment markedly paler. *Legs* dusky; hind tibiae either all dark or with the last two black, the rest also rather dark but gradually paling proximally; hind and middle femora with a broad infuscated band.

MALE.—*Head* dusky brown to dusky buff. Posterior surface dusky grey, sometimes darker towards the upper margins; facial pit grey to ochreous grey; frontal margins shimmering creamy white or yellow white. *Antennae* with the terminal segment *strongly recurved, and clothed with long pubescent hairs*, the longest hairs on the dorsal edge being equal to about three-fourths the width of the segment. *Palpi* long, relatively thin and dark brown, tips slightly darker. Bulb of proboscis pale, with the proximal portion faintly infuscated.

Thorax.—Very dark brown to blackish brown, with very narrow, pale, longitudinal stripes, the sub-median ones being generally sharply and clearly defined, and scarcely broader than the pale transverse suture; pleurae dark brown.

Abdomen.—Blackish brown, with the second segment ochreous brown, there is no median stripe to the remaining segments, but a trace of ochreous brown is faintly visible on the third, fourth and fifth; lateral margins of all the segments narrowly paler than the rest; venter dusky ochreous; sides of the third, fourth and fifth segments, and also the hypopygium, infuscated; stigmata white.

Legs.—Yellowish brown or buff; femora broadly and distinctly infuscated, especially the middle and hind pair; middle and hind tibiae also with a very broad infuscated blotch; anterior tarsi with the tip of the fourth segment black, last segment dark at tip but not black; middle tarsi similar; hind tarsi all dark but the distal half of the third and the two succeeding ones all black; hairs on ventral surface bright golden brown.

The female differs from the male in having the legs decidedly paler; the hind tarsi with the last two segments black, the second and third with the tips only black. The thoracic and abdominal markings, and also the form and strongly pubescent character of the long terminal segment of the antennae are specifically the same as in the male.

Described from four examples, three males and one female. One male was taken at Kasongo, Congo Free State, 6th February, 1904 (Drs. Dutton and Todd), one at Sunyani, Ashanti, 5th March, 1910, and the third at Atroni, W. Ashanti, 16th August, 1910 (Dr. A. Kinghorn). The only female I have yet seen was taken at Odumase, W. Ashanti, 27th April, 1910, by Dr. A. Kinghorn.

Glossina fuscipes, n. sp.

This tsetse fly may be readily distinguished from *Gl. palpalis*, Rob.-Desv., by its much smaller size, by the uniformly infuscated or dusky legs, and the dusky grey thorax. In size it resembles *G. tachinoides*, West, but it is a relatively stouter built insect; and altogether it is most like a dwarfed specimen of *Gl. palpalis* with infuscated legs and a dusky thorax. The genital armature resembles those of *Gl. palpalis* and *Gl. tachinoides* in its general form; but the shape of the superior claspers and the inferior claspers are markedly distinct from those of either of these insects.

Length.—7½ mm.; length of wing, 8 mm.

MALE.—*Head:* Frontal stripe yellowish brown, margins dusky white; ocellar triangle buff, enclosing a very dark brown spot. *Antennae* grey; arista dark brown ventrally. *Palpi* smoky brown, with a paler narrow median line. *Proboscis* with the bulb dark castaneous.

Thorax with a median dark grey rectangular area extending from the front to the scutellum, the sides of which are perfectly straight and parallel, on either side of this grey area are two greyish-black, and somewhat triangular blotches, evidently remnants of those found in other species of Glossina; scutellum dark grey, with a very faint pale grey median line, margins buff; sides of the lateral thoracic depressions dark brown or almost black; pleurae dark grey.

Abdomen resembling that of *Gl. palpalis*, but the bilateral banding faintly pronounced; lateral margins with pale angular areas.

Legs strongly and almost uniformly infuscated (dark grey); femora with the basal and ventral portions ochreous buff; hind tarsi all dark brown or almost black.

Wings.—Anterior transverse vein strongly and suddenly incrassate at its junction with the third longitudinal vein.

HABITAT.—The only example which we possess of this interesting tsetse fly was taken by Dr. Shircore,* at Nimule, Nile Province, Uganda. Fortunately it proved to be a male, otherwise it may have passed as a small dusky form of *Glossina palpalis*.

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* The data here given are taken from Dr Shircore's letter; the actual locality is not stated by him. *R. N.*

DESCRIPTIONS OF A NEW GENUS AND THREE NEW SPECIES OF ANOPHELINE MOSQUITOS

BY

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AND

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(Received for publication 15 *December*, 1910)

DACTYLOMYIA, nov. gen. (Newstead and Carter)

(Figures 1-3)

Head.—Clothed with upright forked scales; palpi rather densely scaled.

Thorax (fig. 2).—With a *distinct and very pronounced cylindrical-shaped tubercle* or finger-like process projecting obliquely from the prothoracic region, mid-way between the dorsum and venter, and arising from the anterior margin. Thorax and abdomen clothed with hairs.

Wings.—With dense lanceolate scales. Judging by the character and distribution of the scales this genus comes near *Anopheles*; but owing to the remarkable structures mentioned above it is easily distinguishable from any other known genus of the *Anophelinae.*

Dactylomyia ceylonica, nov. sp. (Newstead and Carter)

Under pocket lens × 16.

Head.—Black in the centre, white in front; palpi brown, with three white bands and a few spots at the base, the apical band being the narrowest; proboscis with the apical half yellow, except for a minute black band near the apex.

Thorax and abdomen dark, covered with golden hairs.

Legs.—Brown, with the femora, tibiae and metatarsi spotted, the apex of the tibiae with a white spot; tarsi narrowly banded.

Wings.—With two small basal and four large costal spots, and many spots on the wing field; fringe with a large pale spot extending from the middle of the first fork cell to a short distance below the apex of the third long vein; there are other pale areas at the apices of the veins.

Microscopical characters.

Head.—Black, with a tuft of long white scales and several pale bristles projecting between the eyes; the rest clothed apparently witn white, upright forked, scales in front and dark ones behind, but owing to its rather denuded condition we cannot be certain as to the exact nature and distribution of the head scales.

Palpi (fig. 1).—With the basal half clothed with brown scales, with the exception of three small spots formed by white ones; the apical portion with two broad bands of pure white scales, a narrow band of yellowish scales and short golden hairs at the apex.

Proboscis (fig. 1).—Distinctly curved at the base and clothed with deep brown scales on the posterior half, dull yellow ones anteriorly, and immediately before the labella are a few blackish scales, hardly sufficient in number to form a band.

Antennae.—Pale brown, the basal segment bare (? denuded), the first segment with many white scales.

Thorax.—Very dark brown, almost black, with a greyish tinge at the sides; hairs pale ochreous; thoracic tubercle (fig. 2) distinctly cylindrical in shape and gradually rounded in front; it is sufficiently prominent to be seen distinctly with a pocket lens; dark brown, shining on the denuded portion, sparsely clothed at the apex with fine pubescent hairs; sides apparently with minute greyish scales; scutellum dark brown with many long dark border bristles and also several shorter golden ones; halteres pale, the knobs clothed with small white scales.

Abdomen.—Dark brown covered with golden hairs.

Legs.—Deep brown; the femora, tibiae and metatarsi with white spots; tarsi of the first pair with narrow yellowish bands involving both sides of the articulations, last tarsus with dull ochreous scales; hind legs with apical yellow bands, the last tarsal segment, and metatarsus with a distinct apical band; mid legs wanting.

Wings (fig. 3).—Clothed with yellow and black lanceolate scales; costa with two small basal and four large spots, the two median ones being the largest; the first large spot extends evenly on the sub-costa and first longitudinal vein, the second extends evenly on to the sub-costa, but it is represented by two smaller spots on the first longitudinal; the third and fourth spots extend evenly on to the first longitudinal vein, the fourth also being continued to the upper branch of the first posterior cell. The second long vein has a spot just before the cross vein and a rather longer one immediately after; the branches of the cell, besides the spot on the upper branch mentioned above, have several scattered black scales. Third vein with six small sub-equal patches. Stem of fourth long vein completely dark scaled, base of the fork yellowish, upper branch with a black patch immediately after the fork and a few slightly darker scales towards the apex, lower branch with several dark scales, those nearest the base forming a small patch. Fifth vein with a patch near the base and six small, almost equidistant, spots on the remaining portion of the stem and lower branch of the fork, upper branch with four spots, the first one being just after the posterior vein. Sixth vein also with six small equidistant spots. Fringe with pale areas at the apices of the veins, that at the apex of the wing being the largest.

Length.—3·5 mm.

HABITAT.—Trincomalee, Ceylon (E. E. Green).

We are greatly indebted to our colleague Mr. E. E. Green, the Government Entomologist of Ceylon, for this extremely interesting and unique mosquito, and congratulate him on the discovery of such an interesting form. This specimen was presented to the Liverpool School of Tropical Medicine, November, 1907, together with a number of other Anophelines, and until quite recently its true generic position had been overlooked.

Pyretophorus cardamatisi, nov. sp. (Newstead and Carter)

(Figures 4 and 5)

Under pocket lens × 16.

Head.—Black behind, white in front with a projecting tuft of long white scales; palpi long and thin, black, with three white bands, the apical one being the broadest; proboscis *very long and straight*, black with a pale apex.

Thorax.—Grey-brown clothed with whitish scales, with a dark median line and dark lateral ridges.

Abdomen.—Brown, with pale hairs.

Legs.—Brown, with pale articulations.

Wings.—Rather thinly scaled, with two small basal and four large costal spots; fringe with five pale areas.

Microscopical characters.

FEMALE.—*Head* black, with numerous white upright forked scales forming a more or less triangular group in front, the remaining portion covered with black forked scales, with the exception of a few on the posterior lateral margin, which are of a golden yellow colour; projecting between the eyes is a tuft of elongated white scales and several greyish bristles.

Palpi (fig. 4) and *proboscis* very long and thin, the former clothed with dark brown scales, with white bands at the articulations, and with a distinctly broader band at the apex, the latter covered with dark scales, except those on the labella, which are of a dull yellow colour.

Thorax.—Integument of the median dorsal area grey-brown, the sides dark brown, forming two more or less distinct stripes, scales on the pale area greyish white; pleurae paler than the lateral margins of the thorax, almost bare, but with a few scattered grey scales present.

Abdomen.—Pale grey-brown, with dark brown apical margins to the segments, and clothed with greyish hairs.

Legs.—Brown, all the articulations pale.

Wings (fig. 5).—With lanceolate scales on the veins. Costa with two small basal and four large spots, the latter approximately equidistant and sub-equal in length, all extending on to the first longitudinal vein, spaces between the spots clothed with yellowish scales. The scales on the first two-thirds of the stem of the second longitudinal vein chiefly black, the rest of the stem and base of the fork white; tip of upper branch yellow the rest black, lower branch black with the exception of a small area below the costal spot. Third vein almost entirely clothed with yellowish white scales, except at the base and apex, which are covered with black scales. Basal two-thirds of the fourth vein yellowish, a black spot reaching nearly to the cross veins, then another black patch commencing just after the cross veins and terminating at the fork; base of the fork yellowish, upper branch with two nearly equal black patches and a median pale area; lower branch similar. Fifth long vein with a small black spot at the base, rest of the stem light, base of fork pale, upper branch with a few black scales before the cross vein, space near the latter with pale scales, followed by a patch of black scales then white ones, and distally with a few black ones; basal one-third of the lower branch pale, the rest black. Scales on the sixth vein all black with the exception of two small sub-median patches of white ones.

Length.—4 mm.

MALE.—*Palpi* of the typical Anopheline form with three pale bands; the first at the apex of the narrow portion; the second, which is much deeper ventrally, at the apex of the basal segment of the club; and the third at the extreme tip. The remaining characters as in the female.

Length.—3·5-4 mm.

HABITAT.—Athens (Dr. J. P. Cardamatis).

The specimens of this species were presented to this School by Dr J. P. Cardamatis, November 18th, 1907, and we believe that they were captured by him in the summer of that year.

The distinctive characters of this Anopheline are the markedly long, thin, and straight proboscis, the banding of the palpi and the wing markings.

Cellia cincta, nov. sp. (Newstead and Carter).

(Figures 6 and 7)

Under pocket lens × 16.

Head.—White, dark posteriorly; proboscis black, apex yellow; basal portion of palpi dark with dense outstanding scales.

Thorax.—Greyish, with lateral ridges.

Abdomen.—Blackish grey, covered with golden hairs and with outstanding tufts of scales; apical segment with yellowish scales.

Legs.—Femora and tibiae with numerous yellow spots; metatarsi and tarsi with small alternating black and yellow rings, last tarsal joint in all the legs yellow.

Wings.—With five costal spots, the two median ones being much the longest; fringe with six pale areas.

Microscopical characters.

Head.—With a large and more or less triangular area clothed with white forked scales, these scales extend from the vertex to the extreme front, sides clothed with large dense black upright forked scales. Two or more long white bristles project over the clypeus. *Antennae*, basal segment greyish brown; a few of the succeeding segments dark brown, the rest greyish; short hairs silvery grey. *Palpi* (fig. 6*b*) clothed with rough outstanding dusky black scales; articulation of the first and second segments white scaled, in more perfect examples these will in all probability form a distinct ring; the basal portion of the second segment is also clothed with dusky black scales; the remaining portions of the palpi are unfortunately wanting.

Proboscis dusky black, with the labella yellowish white.

Thorax.—Prothoracic lobes clothed with long outstanding blackish scales, these are distinctly spathuliform in shape, with suddenly truncated apices. Thorax greyish brown, with white scales; on the front of the mesothorax these are very narrowly spindle-shaped, and when viewed obliquely appear as narrow curved ones, those on the lower portion of the thorax behind the wings are distinctly spindle-shaped and more dusky-white than those in front; sides of thorax in front greyish. Posteriorly the pleurae are brownish, and with a few scattered white scales. Halteres with a greyish stem, apex darker with a few brown scales.

Abdomen.—Distinctly dark slaty grey; hairs pale; apical margins with dense dark brown outstanding scales, intermixed with these, dorsally, are a few greyish white ones; apical segment clothed chiefly with yellowish white scales; venter blackish, with pale hairs.

Legs (fig. 6*a*).—Middle and hind femora and tibiae very conspicuously spotted with yellowish white scales, so distinctly so, as to appear almost ringed; knees yellowish white; metatarsi and tarsi with distinct and equidistant pale yellowish rings; terminal segment uniformly yellow.

Wings (fig. 7). Clothed with black and yellow lanceolate scales; costa with five spots, all extending on to the first long vein, the third spreading evenly on to sub-costa, but interrupted on the first long vein by a group of yellowish scales near the proximal portion. Second vein with black patches of scales immediately before and after the cross vein, upper branch of the fork except at the base and extreme apex black scaled, lower branch with a pale spot at the base, then a short black patch, and the remaining portion clothed with pale scales, some of which are tinged with black. Third vein entirely yellow scaled, with the exception of a few black scales at the extreme base and apex. Stem of the fourth longitudinal vein with a large spot just before the fork, a few black scales behind the posterior cross vein and at the base; upper branch with two spots, lower branch with one median dark patch. Fifth long vein with a few black scales at the base of the wing, the rest being yellow; upper branch with a small black patch at the base, a larger one at the apex and another in the middle; lower branch completely yellow scaled, except for a small black spot at the apex. Sixth vein with a yellow patch towards the base, the remaining portion black scaled. Fringe with pale areas at the apices of the veins.

Length.—3 mm.

HABITAT.—This interesting Anopheline was captured by Dr. A. S. Donaldson at Broomassie, W. Africa.

It is markedly distinct from any other hitherto described species of *Cellia*, and may be readily distinguished by the very conspicuous and regularly disposed rings on the mid and hind legs. It may be further distinguished also by the rough outstanding scales on the palpi. The only specimen which we possess is in a splendid state of preservation so far as it goes, but, unfortunately, both the anterior legs and the apical segments of the palpi are wanting.

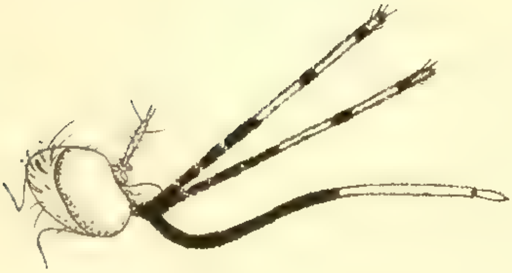

FIG. 1. Head of *Dactylomyia ceylonica.* (Newstead and Carter.)

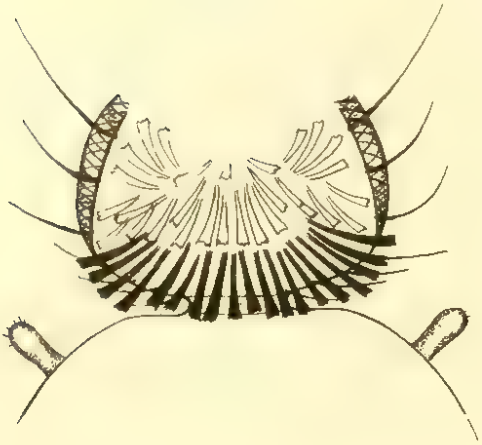

FIG. 2. Portion of thorax, showing tubercles, of *Dactylomyia.* (Newstead and Carter.)

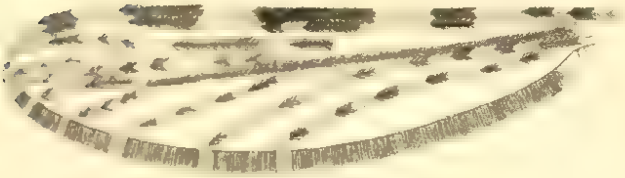

FIG. 3. Wing of *Dactylomyia ceylonica.* (Newstead and Carter.)

H. F. Carter, del.

ERRATA.

Plate XXIV, figs. 6, 7.
For *Cellia annulipes* read *Cellia cincta.*

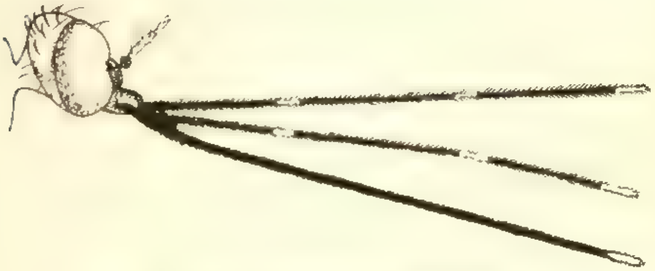

FIG. 4. Head of *Pyretophorus cardamatisi* ♀ (Newstead and Carter.)

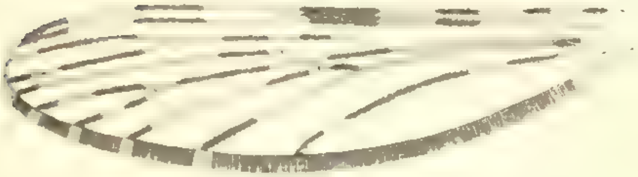

FIG. 5. Wing of *Pyretophorus cardamatisi.* (Newstead and Carter.)

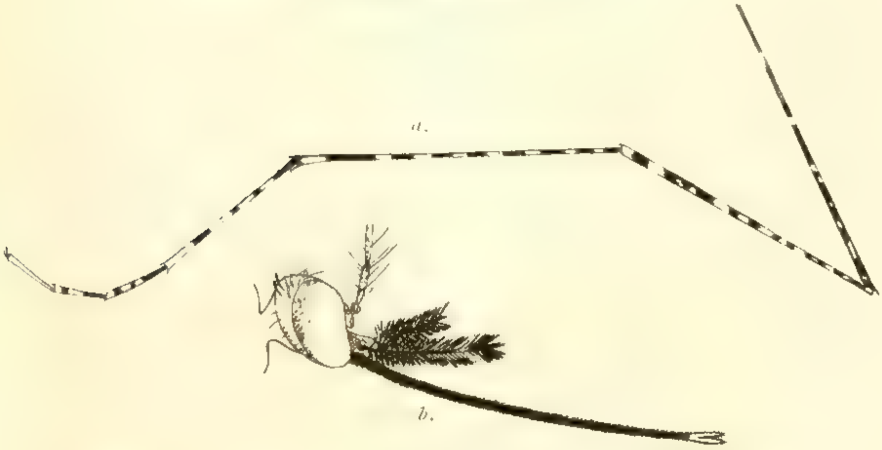

FIG. 6. *a.* Hind leg of *Cellia annulipes.* (Newstead and Carter.)
b. Head of *Cellia annulipes*; anterior portion of palpi wanting

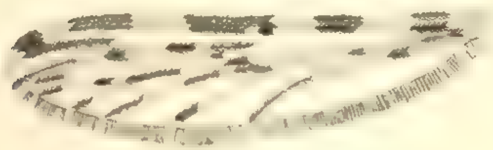

FIG. 7. Wing of *Cellia annulipes* ♀ (Newstead and Carter.)

H. F. Carter, del.

A NOTE ON THE PATHOLOGY OF LESIONS OF THE CORNEA AND SKIN IN ANIMALS EXPERIMENTALLY INFECTED WITH *T. RHODESIENSE*

BY

WARRINGTON YORKE, M.D.

(*From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine*).

Received for publication 2 November, 1910)

As mentioned in a previous paper[*] three goats and a horse infected with *T. rhodesiense* all developed an interstitial keratitis of a remarkably transient character.

GOAT 1. On the sixth day after inoculation the temperature rose to 107° F. and parasites appeared in the peripheral blood, one being found to a field (Zeiss objective DD, eye-piece 4). During the next five days parasites were present in the blood in small numbers, one to from twenty to fifty fields. Subsequently the temperature fell to 103° F., and except on the thirty-first day, when one trypanosome was seen in fifty fields, parasites were not again found in the blood until the day of death, which occurred on the fifty-fifth day after inoculation, when one trypanosome was present in fifty fields.

On the fifteenth day a distinct swelling of the skin and subcutaneous tissue was visible below the eyes and over the nasal bones. This condition became gradually more marked and persisted until the animal's death.

On the seventeenth day it was noticed that the lower portion of the left cornea was slightly milky in appearance. This cloudiness rapidly became more dense, until on the nineteenth day the whole of the lower two-thirds of the left cornea was densely opaque.

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[*] Yorke. 'On the Pathogenicity of a Trypanosome (*T. rhodesiense*) from a case of Sleeping Sickness contracted in Rhodesia,' Ann. Trop. Med. and Parasitol., Vol. IV, p. 351, 1910.

Slight opacity of the right cornea was now apparent. On the twenty-fourth day both corneae were opaque. During the next week the opacity gradually disappeared, until on the thirty-second day the eyes appeared perfectly normal. On the thirty-seventh day the left cornea again became cloudy, and on the thirty-ninth was opaque. The following day slight opacity was observable in the other cornea. The left cornea remained densely opaque and the right slightly cloudy until the death of the animal.

GOAT 2. Parasites were first observed in the blood (one to fifty fields) on the fourth day after inoculation. In the course of the next thirty-three days they were found on ten occasions in very small numbers. During the last nine days no parasites were found. The animal died on the forty-sixth day. The temperature never rose above 104° F. Oedematous swelling of the integuments below the eyes and over the nasal bones appeared on the sixteenth day and persisted until death.

On the thirty-sixth day slight opacity of the left cornea was observed for the first time. The opacity rapidly spread from below upwards, until on the thirty-eighth day the left cornea was densely opaque in its lower two-thirds. On the thirty-ninth day the left cornea was found to be clearing, whilst there was a slight cloudiness of the cornea of the opposite eye. The following day the right cornea was opaque. During the next few days the opacity rapidly disappeared from both eyes, until on the forty-second day the left cornea was clear and the right almost clear. During the last four days the right cornea became slightly more cloudy; the left remained clear until death.

GOAT 3. Parasites (one to fifty fields) first seen in the blood on the eleventh day after inoculation. They were subsequently found in very small numbers on the twenty-first and thirty-ninth days. The animal died on the fifty-fourth day. Temperature remained between 103° and 104° F. Oedematous condition of the skin of the face apparent on the thirtieth day and persisted until death.

Slight cloudiness of the lower portion of the right cornea appeared for the first time on the thirty-ninth day. The opacity rapidly increased in extent and density, until on the forty-second day the whole cornea was densely opaque. The condition persisted until death. The left cornea was unaffected.

In none of the goats was any other pathological condition of the eye observed. The pupils dilated normally with atropine. There was no conjunctivitis nor injection of the sclerotic.

HORSE 1. Parasites were first found in the blood on the seventh day. Subsequently they were present regularly in small numbers until the animal's death on the thirty-eighth day.

Slight haziness of the left cornea was first apparent on the thirty-fifth day. The condition gradually increased in severity, until at the time of death the whole cornea was cloudy.

The right cornea was unaffected. The sclerotic of the left eye was slightly injected.

For the purpose of studying the histological appearances the eyes were removed at the time of death and small portions of the cornea fixed in various fluids, viz., formalin 10 per cent., Flemming's solution and mercuric chloride alcohol. The tissues were embedded in paraffin and the sections stained with Heidenhain's iron alum haematoxylin, Breinl's safranine methylene-blue orange tannin, Giemsa and van Gieson. The best results, so far as the staining of the parasites was concerned, were obtained by staining the tissue which had been fixed in Flemming's solution by either the iron alum haematoxylin or the safranine methylene-blue orange tannin method. In well-fixed sections stained by these methods the trypanosomes stained exceedingly well, and the different portions of the parasites were easily distinguished. On the other hand, in those which had been fixed with formalin, the parasites did not stain, or at the best were very indistinct.

For purposes of description the eight corneae examined may be grouped into four classes.

1. Those which were densely opaque at the time of death, viz., left cornea of Goat 1 and the right of Goat 3. In these the morbid changes were very marked. The pathological conditions in the two were somewhat different. In the left cornea of Goat 1 the lesions were entirely confined to the substantia propria, the anterior and posterior epithelial layers being unaffected. Scattered throughout the substantia propria, which was almost twice as thick as that of a normal eye, were small oedematous patches. These were situated for the most part in the anterior third of the substantia propria. In the distended interlamellar spaces were a few leucocytes (both

polymorphonuclear and mononuclear, and also large numbers of trypanosomes. In the deeper layers of the substantia propria there was marked cellular infiltration and also considerable vascular formation. These areas also extended in places into the anterior third reaching occasionally almost to the anterior homogeneous membrane. Trypanosomes were found scattered throughout this portion of the cornea and in certain spots they existed in enormous numbers.

Appearances somewhat similar to the above have been described by Morax* as occurring in the corneae of dogs and goats infected with *T. equiperdum*.

The state of affairs obtaining in the right cornea of Goat 3 represented a rather more advanced stage. The substantia propria was about three times as thick as that of a normal cornea, and exhibited similar changes to those described in the previous case, viz., markedly oedematous areas lying side by side with patches of intense leucocytic infiltration. There was also some extravasation of red cells from the newly-formed vessels. The morbid processes were not, however, confined to the substantia propria, but had involved the anterior epithelial layer, which in places was separated from the substantia propria by inflammatory exudation. In these regions the epithelial cells were degenerated and stained badly, and at some points were completely disintegrated.

In contradistinction to the previous case only few trypanosomes were found scattered throughout the substantia propria.

II. Those which were slightly opaque at the time of death, viz., right cornea of Goat 1 and of Goat 2 and the left of the horse. Here the lesions were limited to the substantia propria which was slightly increased in thickness. In the deeper layers near the corneo-sclerotic junction there was considerable cellular infiltration and slight vascular formation. Elsewhere the only change visible was a slightly oedematous condition with the occurrence of a few leucocytes and trypanosomes scattered throughout the interlamellar spaces.

III. The left cornea of Goat 2, which after being densely opaque had subsequently become perfectly clear again. The anterior and posterior epithelial layers were normal. The substantia propria was slightly thicker than normal and oedematous in places. Near the corneo-sclerotic junction there was considerable infiltration of polymorpho- and mono-nuclear leucocytes and some new vascular formation, but elsewhere only a few leucocytes were found. No parasites were seen.

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* 'Manifestations oculaires au cours des Trypanosomiases,' Annales de l'Institut Pasteur, 1907, p. 47.

IV. The left cornea of Goat 3 and the right of the horse, which had remained unaffected throughout the disease. In these nothing abnormal was observed microscopically.

A comparable condition of affairs was found to exist in the lesions of the skin of an infected rabbit.

Small pieces of skin were removed from the following different areas:—(*a*) Normal skin of back; (*b*) area recently affected in which the hair was being shed and the skin oedematous; (*c*) bald markedly oedematous area; (*d*) region which had been bald for some weeks and showed some signs of recovery. The thickening of the skin had largely disappeared and there was a slight re-growth of hair.

The methods of fixing and staining were those employed for the cornea.

The histological appearances presented by sections of skin from these different areas may be classified as follows:—

(*a*) No pathological changes and no parasites were found.

(*b*) Large numbers of trypanosomes were found lying in the oedematous interstitial spaces. There was also considerable cellular infiltration.

(*c*) In this region the skin was considerably thickened and markedly oedematous. Enormous numbers of trypanosomes were present. There was great cellular infiltration involving the hair follicles and glands. Many of the hair follicles had completely disappeared.

(*d*) Here no parasites were found. The skin was not distended with fluid and the cellular infiltration was only slight.

Judging from the appearance presented by this series of sections of the cornea and skin the course of events would seem to be somewhat as follows:—

In early lesions trypanosomes are present in the tissues, and as a result there is an oedematous condition of the part, and a more or less marked degree of leucocytic infiltration.

Later as the number of parasites increases the morbid condition becomes more accentuated. Large numbers of leucocytes are poured into the tissue and new vessels develop. After a time the parasites disappear and with their disappearance there is a tendency on the part of the tissue to recovery.

The rapidity with which these processes occurred in the cornea is very remarkable. One can hardly suppose that the changes in the right cornea of Goat 1 and the left of Goat 2, which from being densely opaque were after the interval of a few days apparently normal, had progressed so far as the formation of new vessels; yet on account of the extreme opacity, there must have been very considerable cellular infiltration, although this might in part be explained by the presence of enormous numbers of parasites such as were seen in the left cornea of Goat 1, which was densely opaque at the time of death.

The fact that trypanosomes can multiply so readily in the tissue spaces and at the same time be either entirely absent from the blood, or present in very small numbers only, is one of considerable interest, although the explanation is not very obvious. Perhaps the tissue juices form a more favourable nidus for the growth of the parasites, or possibly in these situations they escape to some extent the action of certain anti-bodies which have been shown to exist in the blood. Whatever the cause may be, the observation illustrates in what manner it is possible for an animal to be heavily infected and at the same time present no parasites in the peripheral circulation.

The drawings were done by Miss A. M. Brookfield.

DESCRIPTION OF PLATES.

PLATE XXV.

Left cornea of Goat 1.—Fixed with Flemming's solution. Stained by Heidenhain's iron alum haematoxylin method. Drawn with Abbé camera lucida, 2 mm. apochromatic objective and No. 8 compensating ocular (Zeiss). Magnification 1,000 diameters.

Considerable oedematous infiltration of the substantia propria. In the distended interstitial spaces are large numbers of trypanosomes. The parasites are cut in various directions, some transversely and others obliquely, whilst others are lying horizontally in the plane of the section.

There is also considerable infiltration of polymorphonuclear and mononuclear leucocytes. Two small blood vessels are seen in the lower portion of the drawing.

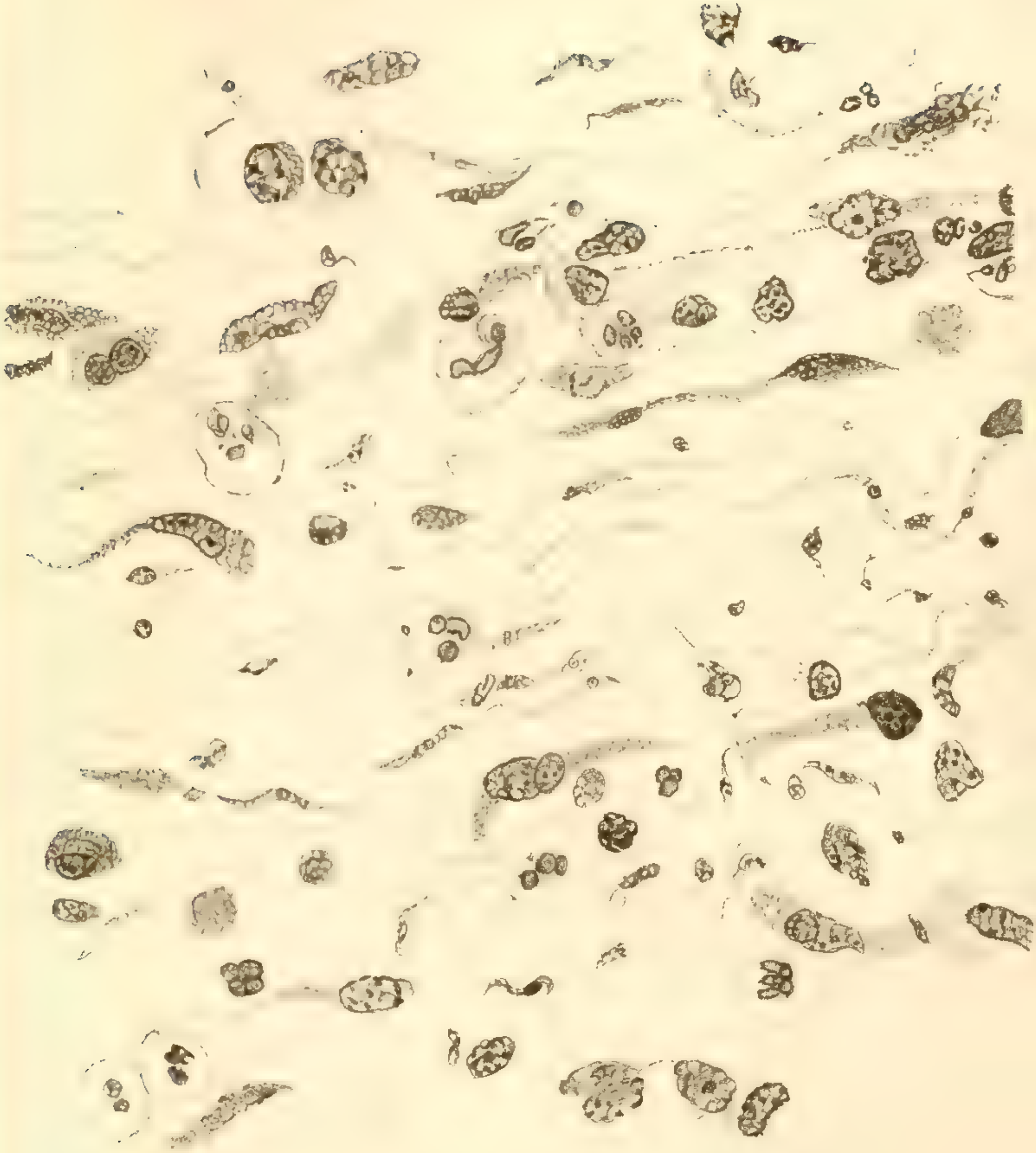
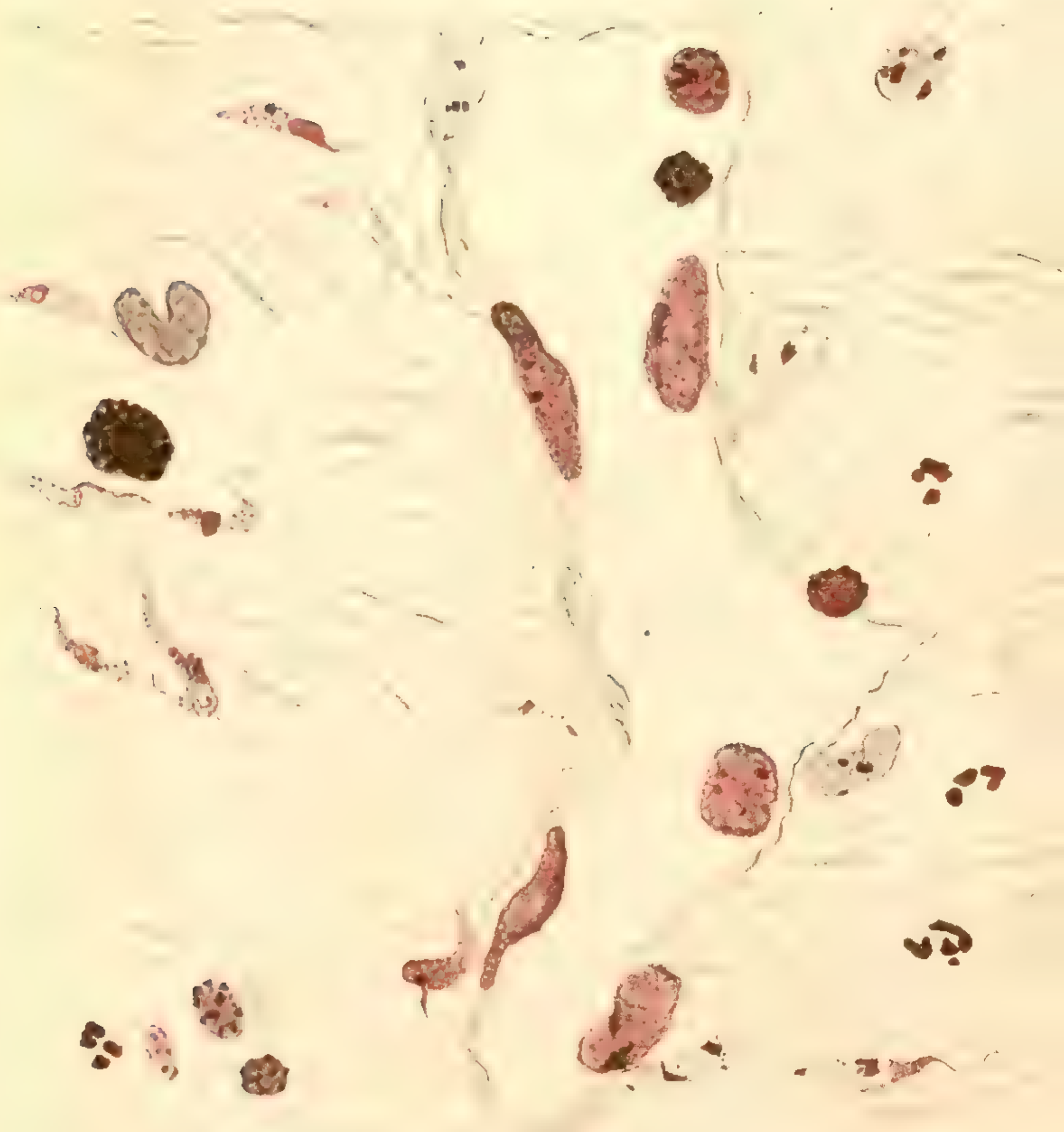


PLATE XXVI.

Right cornea of Goat 3.—Fixed with Flemming's solution. Stained by Breinl's safranine methylene-blue orange tannin method. Drawn with Abbé camera lucida, 2 mm. apochromatic objective and No. 12 compensating ocular (Zeiss). Magnification 1,500 diameters.

The drawing represents a small portion of an area of marked cellular infiltration and vascularisation. A small vessel runs vertically across the field. Trypanosomes, polymorpho- and mono-nuclear leucocytes are seen lying between the fibres of the substantia propria.



A CASE OF SLEEPING SICKNESS STUDIED BY PRECISE ENUMERATIVE METHODS: FURTHER OBSERVATIONS

BY

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AND

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Received for publication 15 *October*, 1910[*]

I. INTRODUCTION

In a previous paper[†] by us, we recorded our observations on this case during two and a half months, and described particularly the regular periodical rises in the numbers of trypanosomes disclosed in the patient's peripheral blood by methodical daily countings extending over that period. Our technique has been detailed in another paper by us on 'Enumerative Studies on Malarial Fever.'[‡] We now record our further observations on the case during two more months—until the patient's death. A chart and a table giving daily details of the observations are attached; and accompanying papers by Drs. H. B. Fantham and J. G. Thomson record studies on animals and on the parasites themselves. We must refer also to a recent communication by Drs. J. W. W. Stephens and Fantham[§] suggesting that the species found in this case may not be identical with *T. gambiense*.

The patient, a strong young Englishman, age 26, weight 154 lbs., was infected in N.E. Rhodesia near the River Luangwa in September, 1909. The trypanosomes were found in his blood in Africa on November 17.

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[*] Reprinted from Proc. Roy. Soc., B, Vol. LXXXIII, p. 187.

[†] Proc. Roy. Soc., July 21, 1910, B, 557.

[‡] Ann. Trop. Med. and Parasit., Vol. IV., No. 3.

[§] *Ibid.*

He was admitted into the Royal Southern Hospital on December 4. Daily estimation of the number of trypanosomes per cubic millimetre of his blood was commenced on February 16, and continued till his death on June 29, a period of 134 days. During that period we never failed to find trypanosomes. The history of the case from February 16 is recorded graphically on the chart.

The patient had glandular swellings and ill-defined erythematous rashes on his legs at various intervals. On April 20 he had a severe attack of vomiting which continued for four days, in consequence he lost 10 lbs. in weight and was never again so well. He remained in bed onwards till his death. He became progressively more drowsy, with intervals of more or less comparative brightness. His memory and mental powers failed steadily. On May 7 he developed a marked neuritis due to the atoxyl, and every dose seemed to aggravate the neuro-retinitis produced by the previous administration of this drug. His eyes were examined weekly by Dr. Hamilton, oculist to the hospital.

On May 10 both legs became markedly oedematous and remained so till his death. On May 13 he became much worse and developed paralysis of his sphincters. He seemed about to die, but on May 20 a sudden remarkable improvement took place. Soon, however, he again commenced to grow steadily worse.

On June 25 he developed pleurisy and pneumonia. He died on June 29.

Post mortem.—Performed twenty-two hours after death:—

Brain.—Membranes thickened and white. Vessels congested. Cerebrospinal fluid increased.

Left lung.—Broncho-pneumonia.

Left pleural cavity.—Contained one pint of yellow pussy fluid

Spleen.—Soft and much enlarged; weight, 33 ozs.

Liver.—Enlarged; weight, 6 lbs.

Blood.—No trypanosomes could be found. Leucocytes, 17,000 per cubic millimetre. Polymorph excess.

Smears of bone marrow, spleen, kidney, mesenteric glands, and cerebrospinal fluid, all showed large numbers of small and large mononuclear cells, but no trypanosomes could be detected.

II. CONTINUANCE OF THE PERIODICAL CYCLE

The cycle recorded by us till April 30 in our previous paper continued till his death. From February 22 till June 25 there occurred nineteen rises in the numbers of trypanosomes in his peripheral blood. This gives an average of six and a half days between the heights of each rise.

The shortest period between two successive heights is four days, as recorded between April 18 and 22, and again between June 3 and 7. This exceptionally short interval was due, we think, to artificial means, as we shall explain later under vaccine treatment. The longest period recorded between two successive heights is eight days, while the usual period between the heights seems to be seven days. It will be observed that the rise and fall is sudden, and that the height is not maintained.

While the number of the parasites was increasing rapidly, we observed many forms with double nuclei and blepharoplasts (dividing forms). During the interval between the rises, few of these forms were present. This would seem to indicate that the sudden rise is due to an active multiplication of the parasites.

On April 28 the numbers increased from 200 to 1,536 in twenty-four hours, and on June 19 they increased from 100 to 1,500 in twenty-four hours, suggesting that in our patient they were capable of dividing and sub-dividing three to four times in twenty-four hours.

The natural fall is even more sudden than the rise. On April 29 the parasites fell from 1,536 to 100 per cubic millimetre of blood in twenty-four hours.

Except on April 5 and 6 only one observation was made daily, so that obviously we may have missed the highest points reached in the various rises. The low levels were, as a rule, maintained for three or four days, forming **U**-shaped bends as recorded in the chart. The lowest number recorded was 8 per cubic millimetre of blood, while the highest reached 1,536 per cubic millimetre of blood.

It is worthy of note that there is a tendency for a high rise to be followed by a low one. This is not without exception, but the three highest rises recorded were followed by the three lowest rises.

III. CORRELATION BETWEEN THE CYCLE AND THE AMOUNT OF FEVER

From the chart one can observe that almost invariably the temperature tends to be higher during the height of the cycle, and that between the rises the temperature keeps lower.

In the table average daily temperatures are recorded. These figures were obtained by taking the average of the four-hourly temperatures observed during the twenty-four hours of each day. We think that this figure gives a fair representation of the amount of fever for the day, and in this table it may be noted that the average daily temperature rises coincidently with the increase of trypanosomes and falls with their fall.

IV. OTHER CLINICAL OBSERVATIONS AND CORRELATIONS WITH THE CYCLE

The pulse rate ranged all through this case from 90 to 120 per minute. During the height of the cycle it tended to increase coincidently with the increased amount of fever. The respiration rate ranged from 20 to 28 per minute, tending also to increase with the rise of the trypanosomes.

With regard to the bowels there is little to note. Their action was easily regulated with small doses of cascara. It was very noticeable that the patient was more drowsy, and tended to have headaches, during the commencement of the cyclical rise, but these symptoms abated at the extreme height and during the subsequent fall of trypanosomes.

The lymphatic glands in the neck, axilla, groin, and popliteal space were more or less always swollen, but this condition was greatly aggravated at intervals, accompanied with marked tenderness, though only sometimes was this coincident with a trypanosome increase.

The urine throughout remained clear, usually slightly acid and without deposits. The specific gravity ranged from 1010 to 1026.

On March 15 he had a diffuse ill-defined erythematous rash over his left leg, occurring just before the height of a trypanosome increase.

V. CORRELATION BETWEEN THE TRYPANOSOME CYCLE AND THE BEHAVIOUR OF THE LEUCOCYTES

Previous to April 23 we made several leucocyte counts with the Thoma-Zeiss apparatus, but were unable to find anything definite. It was only when we made counts daily at the same hour that we were able to find a definite leucocyte change corresponding to the parasitic cycle.

We counted the leucocytes by means of the thick film as referred to in our paper on malaria, and were astonished at the remarkable variations. We found no such variations by our method in normal persons. Simultaneous examination of thin blood films also showed that a remarkable leucocytic variation did take place, corresponding to each rise of trypanosomes.

Daily differential counts were also made in thin films stained with Giemsa, in which we distinguished only between mononuclears and the so-called polymorphonuclears. In these we never counted less than from 300 to 500 leucocytes to ensure greater accuracy. We thus estimated daily the total leucocytes and the total numbers of polymorphs and mononuclears per cubic millimetre of blood, as shown in the chart and table.

From April 23 till May 20 it is clearly seen that coincident with each rise in the number of trypanosomes there is a marked increase in the total leucocytes, and this increase is due more to mononuclears than to polymorphonuclears.

When the trypanosomes begin to increase in number the leucocytes increase also, more especially the mononuclears. When the trypanosomes have reached their height there may be a fall in the number of leucocytes, but this is followed by a still higher rise during the fall of the trypanosomes. The leucocytes would seem to reach their highest numbers on about the third day after the height of the parasitic cycle. They then decrease rapidly for about three days to normal or much lower. When this occurs the trypanosomes again commence to multiply, and the leucocytes again increase also, so that we have both a parasitic and a corresponding leucocytic cycle.

We did not attempt to differentiate between large mononuclears and lymphocytes, but it seemed to us that both took part in the total mononuclear increase. We further observed that during and immediately after the parasitic fall the large mononuclears were filled with vacuoles and a reddish *débris* (stained Giemsa). Many of these vacuolated mononuclear cells were of great size (30 to 40 microns in diameter). We are inclined to think that this vacuolation with reddish stained *débris* may be an indication of the ingestion of trypanosomes, although we have never observed any definite trypanosome structure within them.

F. W. Andrewes, in the Croonian Lectures,[1] points out that an intravenous injection of bacteria causes a temporary diminution in leucocytes, followed by a marked increase, this fall and rise being chiefly due to the so-called polymorphonuclears, the mononuclears taking little part in the phenomenon. In this disease, as in malaria it would appear that there is a diminution followed by an increase, chiefly of the mononuclears. This tends to suggest that the polymorphonuclear leucocytes react chiefly to bacterial infections, while the mononuclears would seem to react chiefly to protozoal blood infections (D. T).

It is interesting to note that on May 20 there was a very marked mononuclear increase, coincident with a fall of trypanosomes, and accompanied by a remarkable improvement in the clinical condition of the patient; and again on June 24 the mononuclear excess changed into a very marked polymorph excess. This was coincident with the onset of pleurisy and pneumonia, from which the patient died.

In connection with the leucocyte cycle, the highest numbers recorded were 50,000 per cubic millimetre of blood, whereas the lowest was 2,800 per cubic millimetre of blood. In the latter part of the chart the leucocytic graph is altered by the injection of leucocytic extract, and by the development of an abscess and pneumonia.

The following quotation from the 'Sleeping Sickness Bulletin,' 1908, No. I, p. 5, is of interest:—'Dr. W. Thomas found that 'after an injection of atoxyl a change in the parasites became 'noticeable between the fourth and the fifth hour. They became 'sluggish and much altered in appearance, and at the same time 'there was a noticeable increase in the leucocytes. About the seventh 'hour there was a great diminution in the number of trypanosomes.

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[1] Lancet, July, 1910

'and a coincident increase in the leucocytes, notably the phagocytes. 'At the eighteenth hour parasites were absent and could not be 'found, even after blood centrifugalisation. Instances of 'phagocytosis were observed on three occasions.'

Thomas and Breinl tried the effect of hyperleucocytic agents without good effects, but remark: 'It is quite evident that the 'leucocytes play a rôle in the decrease of parasites.'

We think that it is possible that on some occasions, at least, Thomas may have happened to give atoxyl at the time when the leucocytes were increasing and the parasites were diminishing, as we have shown to occur naturally.

VI. THE HAEMOGLOBIN AND RED CELLS

During the course of the disease, from February till the patient's death in June, the haemoglobin percentage fell more or less steadily from 85 to 70 per cent. We could not detect any variation in the amount of haemoglobin corresponding to the parasitic cycle.

The number of red cells was deficient (3,800,000 in March; 2,800,000 in June). Their numbers were not estimated frequently.

VII. THE EFFECTS OF TREATMENT BY VARIOUS DRUGS

The crucial test of a curative treatment in this disease would naturally be the effect of the treatment on the numbers of parasites, especially in our case, where the numbers were estimated daily.

In testing the value of various therapeutic agents, we have therefore taken the graph of the number of parasites as the indicator of the efficacy of that agent. We would like also to point out that in estimating the effect of treatment by this method, the blood must be examined every day, otherwise the effects recorded might be erroneous. If a drug be given at the height of a parasitic rise, and the blood examined next day or a few days later, the number of parasites would naturally be much less; to conclude from this that the drug has caused this diminution might be quite erroneous. It is obvious that no conclusion should be drawn until the numbers have been estimated daily for several weeks.

We think it may be possible that a drug such as atoxyl may by chance have been given, sometimes, just as the parasites were naturally about to fall, and the rapid diminution attributed to the drug.

It has been stated that atoxyl did not always cause a disappearance of the parasites, but that sometimes instead they even increased in numbers; and further, that it seemed to have a more marked trypanocidal action when the parasites were very numerous.

These statements can be understood in the light of the natural cycle.

Again, if the trypanosomes increase in number immediately after the administration of a drug, one cannot at once conclude that this drug is of no value as a trypanocide.

The Effect of Atoxyl

From February 16 till April 5 atoxyl was not given, on account of its injurious effect on the patient's eyes. On April 5 we, however, injected a dose of four grains at the commencement of a natural rise. The blood was examined every two hours afterwards and showed that the trypanosomes were increasing in numbers. Twenty-four hours later there was a further marked increase. Next day again, as we would have expected to occur naturally, there was a decided diminution in their number.

On May 2 atoxyl was again given, though it was found that every dose aggravated the eye condition. From May 2 till June 20, thirty-two grains of atoxyl in all were administered at intervals, as noted on the chart. On comparing the graph where no atoxyl was given, and where it was administered, no very appreciable difference in the number of trypanosomes can be detected. We cannot, however, conclude that the atoxyl has no trypanocidal effect, as had it not been given the trypanosomes may possibly have been much more numerous during the latter than in the earlier part of the chart; and further, this particular strain of trypanosomes seems to have been very virulent.

Our doses of atoxyl in any case were rather small. It would appear to us, however, that atoxyl cannot be considered a specific in human trypanosomiasis, as quinine is in malaria. In our cases of malaria quinine never failed to diminish, markedly, the number of asexual parasites. Moreover, atoxyl compared with quinine is a dangerous drug.

Quinine and Methylene Blue Treatment

Quinine 30 grains daily, combined with methylene blue, 12 grains daily, was administered by the mouth from February 21 till March 9, and again at other intervals as noted on the chart. No marked trypanocidal effect can be noticed.

During treatment with these drugs, however, as also with atoxyl, the patient's face, which had before been puffy and oedematous, especially about the eyelids, became more firm and clear cut. The eyelids became again oedematous soon after these drugs were withheld. It would seem, therefore, that quinine and methylene blue, as well as atoxyl, had some beneficial effect clinically. The following drugs were also given without apparent results: –

(*a*) *Trypsin and Amylopsin Injections.*—20 min. of each daily.

b) *Succinamide of Mercury Injection.*—One-fifth grain almost daily.

(*c*) *Izal Oil.*—8 min. daily, by mouth.

(*d*) *Trypan Red.*—By the mouth in doses of 0·5 grain, 1 grain, and 1 grain respectively on three successive days. No albuminuria resulted, but the drug was stopped on account of the development of severe vomiting.

(*e*) *Potassium Iodide.*—30 grains daily, by mouth.

We beg to apologise for complicating the case with so many treatments, but owing to the unsuitability of atoxyl in the patient other treatment was a clinical necessity.

VIII. EFFECT OF TRYPANOSOME 'VACCINES

On April 19 we commenced to give our patient subcutaneous injections of so-called vaccine. This vaccine was obtained from the blood of a rat inoculated from our patient. The rat was killed when the parasites were extremely numerous (500,000 per cubic millimetre of blood). The blood was then drawn from its heart aseptically, and mixed with an equal volume of normal saline. The red cells were allowed to settle, and the supernatant fluid pipetted off. This latter contained most of the trypanosomes and the number per cubic millimetre was estimated by the thick film method. It was then sterilised by heating to 55° C. for half-an-hour, and by adding trikresol so that it contained 0·2 per cent.

The later vaccines which we commenced to use from May 17 were simply the blood of rats, taken when the trypanosomes were very numerous. They consisted of dead trypanosomes, red cells, leucocytes, and serum. The injection of these vaccines produced no local reaction, even in doses of 100,000,000 trypanosomes, nor were we able to detect any definite temperature reaction.

We are inclined to think that the chief result of these injections of dead trypanosomes was a stimulation of the reproductive powers of the living trypanosomes. This point, however, requires further elucidation. We found that after an injection of our vaccine, the next trypanosome rise usually occurred before it was naturally due.

On April 9, 9,000,000 dead trypanosomes were injected; the next trypanosome rise reached its height on the seventh day 20,000,000 were again injected on April 13, and the following rise was completed on the sixth day. 40,000,000 were then injected and the next rise was completed on the fifth day.

The patient's trypanosomes, also, which were rising to successively smaller heights, continued to diminish further in number, after two more injections of 10,000,000 dead trypanosomes on April 23 and 25. On April 28, however, an injection of 10,000,000 was given when the trypanosomes were increasing, and that rise was the highest recorded. This treatment was then stopped for some time.

It seems that the effect of these so-called vaccines, if injected immediately after the natural fall of the parasites, is a reproductive stimulation of the parasites, causing the next rise to occur sooner than was natural; and this premature rise tends to be less high than it probably should have been in the natural course of events If, however, the vaccine be injected during the natural rise of the trypanosomes, it stimulates their reproduction, causing a very high and rapid rise, and the subsequent fall is also very sudden.

We confirmed these surmises later. On May 17 an injection of 30,000,000 dead trypanosomes was given, with the result that the following rise was completed on the sixth day.

Another injection of 50,000,000 on May 30 was followed by a rise at the normal time. A large dose of 100,000,000, injected on June 4, however, scarcely allowed the parasites any time to diminish, so that they completed their next rise on the fifth day, followed by a sudden fall. Thus it would seem that the normal period of seven days between the rises of parasites may be shortened to six, five, and even four days, by an injection of dead trypanosomes.

Doses of vaccine up to 100,000,000 seemed to cause no harm. On the contrary, during the period from the 7th to the 28th of April, when small doses were given, the temperature was more uniform, and more near to normal than it had ever been before or after.

Again, from the 14th to the 19th of May, the patient was extremely ill, almost comatose, with a high temperature, and like to die. On May 17 he had vaccine (30,000,000), followed on May 19 by an injection of 10 c.c. of leucocytic extract. The improvement on May 20 was remarkable, both mentally and physically. Of course, at that time he had atoxyl and nuclein to increase the leucocytes, hence it is almost impossible to tell which was the potent factor. Against atoxyl having caused this good result, we may point out that it had no such effect either before or since.

As already stated, this marked improvement was coincident with a large increase of mononuclear leucocytes. We tried again to repeat this success by injecting vaccine (100,000,000 trypanosomes) on June 4, three days before the height of the parasitic rise, and leucocytic extract at the height of the rise, but the result was not so good. (*Vide* paper by Ross and J. G. Thomson.†)

IX. THE EFFECT OF SUBCUTANEOUS INJECTIONS OF LEUCOCYTIC EXTRACT

Thinking that the fall in the number of leucocytes might be a factor in the rise of trypanosomes, we determined to keep up the numbers of leucocytes when they were falling. Consequently, we tried an injection of nuclein (25 min.), but this did not seem to cause much increase of leucocytes. Yeast, 15 grains daily (by mouth), was also tried. It seemed to cause some increase, but not marked.

At this time Dr. Moore Alexander, pathologist to the hospital, suggested that we should try leucocytic extract. He thought that this extract might contain certain substances which would be deleterious to the trypanosomes, or which would neutralise their endotoxins. He was good enough to prepare it for us by injecting Mellin's food into the pleural cavities of rabbits. The rabbits were then killed, and the accumulation of leucocytes taken from the pleural cavity, and extracted with sterile distilled water.

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† Proc. Roy. Soc., Vol. LXXXIII, 1911, p. 227.

To our surprise, the effect of an injection of this leucocytic extract was to produce a very great increase of leucocytes in the blood on the day following the injection. This was almost the invariable result. In our opinion, it seems to be a far more powerful promoter of leucocyte increase than yeast or nuclein.

We tried to utilise this discovery in two ways:—

(i) To help the natural leucocyte increase by injecting the extract just at the height of the trypanosome rise. We did this on May 19. Next day, as we have mentioned, the patient showed a remarkable improvement. We were unable, however, to get such a happy result again.

(ii) By injecting the extract a day or two before the rise of parasites, we thought we might prevent that rise by causing an early increase in the leucocytes.

On May 31 10 c.c. of the extract were injected, producing next day an increase of leucocytes up to 20,000 per cubic millimetre of blood, but they diminished next day to 2,800 per cubic millimetre, and the increase of trypanosomes occurred all the same.

Later, just before the patient's death, we tried the effect of 1 c.c. doses of the extract daily. This seemed to keep the leucocyte count high, but unfortunately the results were complicated by the patient developing an abscess at this time, and later by the development of pleuro-pneumonia.

Further investigation with this substance, and with vaccine, would require to be made before coming to any definite conclusions. Such an investigation we think would lead to further knowledge regarding the natural balance of immunity between the body and the parasites.

It appears to be a law (at least it seems to hold good, we think, with leucocytes and trypanosomes) that the extract of dead animal cells stimulates the corresponding live cells to increase in numbers. This would appear to support the hypothesis put forward by Dr. H. C. Ross, that the extracts of dead tissues promote the proliferation of living cells.*

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* Brit. Med. Journ., June 11, 1910.

X. CONCLUSIONS REGARDING THE NATURE AND CAUSE OF THE TRYPANOSOME CYCLE

The true explanation of this phenomenon must be of extreme importance, not only as regards the treatment of this disease, but also, we think, with regard to the problems of immunity in general.

Before stating our views we cannot do better than quote the following extract from the 'Sleeping Sickness Bulletin,' 1909, No. 12, p. 485:—

'*Des Causes des Crises Trypanolytic et des Rechûtes qui les suivent.*
'A MASSAGLIA. "Comptes Rendus de l'Academie des 'Sciences," Octobre, 1907.

'In some species of animals there are no crises. The 'trypanosomes increase in a progressive regular manner. In others 'the trypanosomes, after an increase, suddenly diminish to such an extent sometimes that they cannot be found in the blood. They 'soon reappear. Massaglia endeavoured to find out the cause of 'the crisis and the subsequent relapses. . . . *Conclusions:* 'Trypanolytic crises are due to the formation of anti-bodies in the 'blood. A few parasites escape destruction, because they become 'used or habituated to the action of these anti-bodies. These are 'the parasites which cause the relapses. Since the trypanosomes 'become more and more used to the anti-bodies the subsequent 'crises become less marked. . . . Thiroux's suggestion of a 'balance between anti-bodies and parasites is an interesting one 'and has some facts in its support. It would serve to explain the 'cause of real or apparent cures mentioned in Bulletin No. 5, 'pp. 193 and 195.'

Here it is clear that Massaglia has observed and has tried to explain the occurrence of these natural rises and falls of trypanosomes. Our opinions on the subject are as follows:-

(1) *The cycle is not due to an unconditional cyclical development of the parasites as is the case in malarial fever.*—If it were so then one would expect the cycle to be more regular, and one would not expect its time to be altered by vaccine injections. The time between the rises varies not only in the same individual, but in different animals. This would appear to suggest that the cycle is not due to a definite parasitic development as in malaria, but is

merely a question of a struggle between the defensive powers of the infected body and the aggressive powers of the trypanosomes.

The more susceptible the animal the shorter is the period between the rises as seen in the case of rats, in which the cycle is almost lost Massaglia evidently failed to observe the slight remissions in the rat.

The greater the resistance of the infected animal the longer is the cyclical period, as in guinea-pig and man.

By an 'unconditional cycle' we mean one of which the *period* is not affected by the resistance of the host or by therapeutic agents – as, for instance, that of *Plasmodium*. By a 'conditional cycle' we mean one which is so affected as in the present case.

(ii) *The increase of parasites is due to their sudden and active multiplication.*—The presence of numerous dividing forms during the cyclical rise would seem to support this statement.

(iii) *The multiplication of trypanosomes is extremely rapid.*—As before stated they seem capable of dividing in man three to four times in twenty-four hours.

In rats the rate of multiplication would seem much greater, as many as ten divisions or generations may occur in twenty-four hours.

(iv) *The rate of multiplication depends on the suitability of the infected blood to the parasite.*—The blood of rats seems more suitable to the parasite than the blood of man and guinea-pigs.

Also this suitability would appear to vary from time to time in the same animal. In our case there occurred high and low rises in the numbers of trypanosomes. Moreover the three highest rises recorded in the chart were followed by the three lowest rises recorded. This is a very significant fact. It would appear to indicate that a high rise sets up some reactionary condition. This reaction, if great, not only causes the decrease of trypanosomes but extends its influence so far as to reduce the next rise.

(v) *The fall in number of parasites is not due to the toxins they develop.*—In man the numbers may reach only 200 per cubic millimetre of blood and then they diminish, whereas in rats they may reach over 200,000 per cubic millimetre of blood and yet continue to rise, and the toxins in the latter case must be much more abundant than in the former.

It would seem, therefore, that the explanation may be reduced to some of the following causes, all of which may come into play.

(i) *The increase of trypanosomes is due to their active multiplication*, the rate of multiplication depending on the following conditions:—

(*a*) The liberation of a reproductive stimulant from the dead trypanosomes of the previous fall.

(*b*) The small number of leucocytes, especially mononuclears.

(*c*) The habituation of the trypanosomes to their anti-bodies.

(*d*) The absence or the diminution of anti-bodies to the trypanosomes.

(ii) *The decrease of trypanosomes is due to their rapid death and to a cessation of multiplication*, probably depending on the following conditions:—

(*a*) The presence of anti-bodies in the serum.

(*b*) The large increase of leucocytes, especially mononuclears.

(iii) *The trypanosomes remaining between the rises are resistant forms*, and tend to become spherical, especially in the internal organs. (*Vide* H. B. Fantham.*)

These resistant spherical forms also occur after atoxyl treatment (Moore and Breinl).

N.B.—The reference to the virulence of this trypanosome and to the cycle in rats and guinea-pigs is taken from the paper on enumerative studies in rats, guinea-pigs, and rabbits by H. B. Fantham and J. G. Thomson.†

The virulence of this strain was also noticed by L. E. W. Bevan and Malcolm Macgregor ('Journal of Comparative Pathology and Therapeutics,' June, 1910), who inoculated animals from our patient before he left N.E. Rhodesia.

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* Ann. Trop. Med. and Parasit., Vol. IV, pp. 465-485; also Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227.

† Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463; also Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211.

TABLE OF DAILY DETAILS

W.A. *Sleeping Sickness.* Infected September, 1909; admitted December, 1909; age 26; male; weight 154 lbs.; died June 29, 1910

NOTE. The parasites are given per c.mm. The temperatures are recorded in the Haematothermic Fahrenheit scale, which is the Fahrenheit scale minus 95° multiplied by 10. The leucocytes are given in 100's per c.mm.; and the haemoglobin in Tallqvist's scale.

	February													March		
Date	16	17	18	19	20	21	22	23	24	25	26	27	28	1	2	3
Number of Tryps. per c.mm.		166	195	140	296	687	800	500	254	130	70		124	570	606	70
Maximum temp., H.F.	60	74	60	40	58	56	54	50	30	40	56	52	56	42	54	36
Average daily temp., H.F.	49	57	46	35	39	44	39	31	29	27	35	28	35	37	35	29
Total Leucocytes, in 100's	56	44	39	50	53	—	—	—	62	—	—	—	99	—	119	—
Polymorphs, in 100's	34	26	26	—	—	—	—	—	36	—	—		53	—	66	—
Mononuclears, in 100's	22	18	13	—	—	—	—	—	26	—	—		46	—	53	—
Mononuclears per cent	39	44	36	—		—	—	—	42	—	—		47	—	45	—
Hb. per cent. (Tallqvist)	R.B.C., 3,800,000			—		—	—	—		—	—		—	—	R.B.C., 3,800,000	—
Treatment	Atoxyl, 2 gr			HgCl₂, ¼ gr.	-	Quinine, 30 gr. Meth. blue, 50 c.c.	*et seq.*			—	—			—		—

	March															
Date	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Number of Tryps. per c.mm.	26 ?	12	40	204	1256	1400	472	152	32	76	160	220	320	68	180	132
Maximum temp., H.F.	34	38	36	50	46	34	38	38	36	34	44	46	38	42	40	36
Average daily temp., H.F.	24	24	25	33	33	28	19	27	25	22	32	33	32	26	32	27
Total Leucocytes, in 100's				—			—		156				31	47		73
Polymorphs, in 100's			—		81		—	—	113				17	24		43
Mononuclears, in 100's				—	35				43				14	23		30
Mononuclears, per cent.		—	—	—					34				46	40		41
Hb. per cent. (Tallqvist)		—	85	85	80				85		85	85	85	75	80	82
Treatment									Quinine stopped					Tryp. red, 5 gr. $HgCl_2$, ¼ gr.	Tryp. red. 15 gr.	Tryp. red. 15 gr

	March												April			
Date	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4
Number of Tryps. per c.mm.	32	24	100	622	1136	322	68	72	32	188	748	1016	144	52	52	40
Maximum temp., H.F.	32	32	40	52	44	38	32	38	28	46	50	42	60	48	32	32
Average daily temp., H.F.	27	21	31	40	37	29	26	24	23	31	37	30	31	35	19	25
Total Leucocytes, in 100's	—	62		45	73	99	83	56	80	47	41	100	47	81	53	76
Polymorphs, in 100's	—	38	—	29	28	57	34	23	47	25	16	?	21	36	21	37
Mononuclears, in 100's	—	24		16	45	42	49	33	33	22	25	?	26	45	32	39
Mononuclears, per cent.	—	40	–	35	62	43	60	59	41	46	62	?	55	56	60	51
Hb. per cent. (Tallqvist)	85	85	85	82	85	85	85		85	85	85	80	80	80	80	80
Treatment		–	—	Trypsin, amylopsin	*et seq.*	–	Vaccine, 1,000,000				Quinine, 20 gr.	*et seq.* Izal oil, 8 min	Izal oil, 8 min.	Izal oil, 8 min.	—	

	April													
Date	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Number of Tryps. per c.mm.	212	680	1164	340	116	172	416	556	650	192	96	172	116	362
Maximum temp., H.F.	52	46	40	34	32	30	40	40	40	30	37	38	40	4
Average daily temp., H.F	37	28	27	23	19	21	33	26	25	21	25	25	24	21
Total Leucocytes, in 100's	61	56	89	59	84	53	40	70	62	81	52	?		
Polymorphs, in 100's	33	34	44	35	59	22	19	45		41	32	?		
Mononuclears, in 100's	28	22	44	24	25	31	21	25		40	20	?		
Mononuclears, per cent.	46	40	50	41	30	58	53	36	—	49	38	47	49	48
Hb. per cent. (Tallqvist)	85	85	85	80	80	80	80	80	85	85	85	85		85
Treatment	Atoxyl, 4 gr.	—			Vaccine, 9,000,000	—	—		Succ. Hg, ¼ gr	Atoxyl, 2 gr.	Atoxyl, 2 gr Vaccine, 2,000,000			Succ. Hg, ¼ gr

	April											
Date	19	20	21	22	23	24	25	26	27	28	29	30
Number of Tryps. per c.mm.	336	90	72	244	156	164	88	48	14	200	1536	12
Maximum temp., H.F.	36	32	52	44	34	32	38	38	32	32	52	42
Average daily temp., H.F.	23	22	31	34	27	24	27	27	24	25	32	3
Total Leucocytes, in 100's	100		—	—	100	130	100	72	120	240	108	140
Polymorphs, in 100's	52	—		—	60	78	70		64	144	52	66
Mononuclears, in 100's	48		—	—	40	52	30		56	96	56	74
Mononuclears, per cent.	48	36	31	52	40	40	30		46	40	52	53
Hb. per cent. (Tallqvist)	85		80	85	80	80	80	80	85	80	85	
Treatment	—	Quinine stopped. Vaccine, 40,000,000			Vaccine, 10,000,000	—	Vaccine, 10,000,000			Vaccine, 10,000,000		Succ. Hg, ½ gr

May

Date	1	2	3	4	5	6	7	8	9	10	11
Number of Tryps. per c.mm....	64	64	68	76	292	8	40	108	56	64	8
Maximum temp., H.F.	38	34	40	48	44	46	46	34	54	44	46
Average daily temp., H.F. ...	24	28	30	34	32	24	27	26	35	25	31
Total Leucocytes, in 100's ...	500	110	53	73	210	174	230	350	150	75	62
Polymorphs, in 100's	265	70	33	4[illegible]	101	87	138	168	63	25	25
Mononuclears, in 100's	235	40	20	33	100	87	92	182	87	50	37
Mononuclears, per cent.	47	36	38	45	52	50	40	52	58	66	59
Hb. per cent. (Tallqvist)	85	80	80	80	8	80	80	80	80	80	8
Treatment	—	Atoxyl. 2 gr.	Succ. Hg. $\frac{1}{5}$ gr		Succ. Hg, $\frac{1}{5}$ gr. Atoxyl, 2 gr.	-	Succ. Hg. $\frac{1}{5}$ gr.	—	Succ. Hg, $\frac{1}{5}$ gr. Quin. et Meth blue.	—	—

May

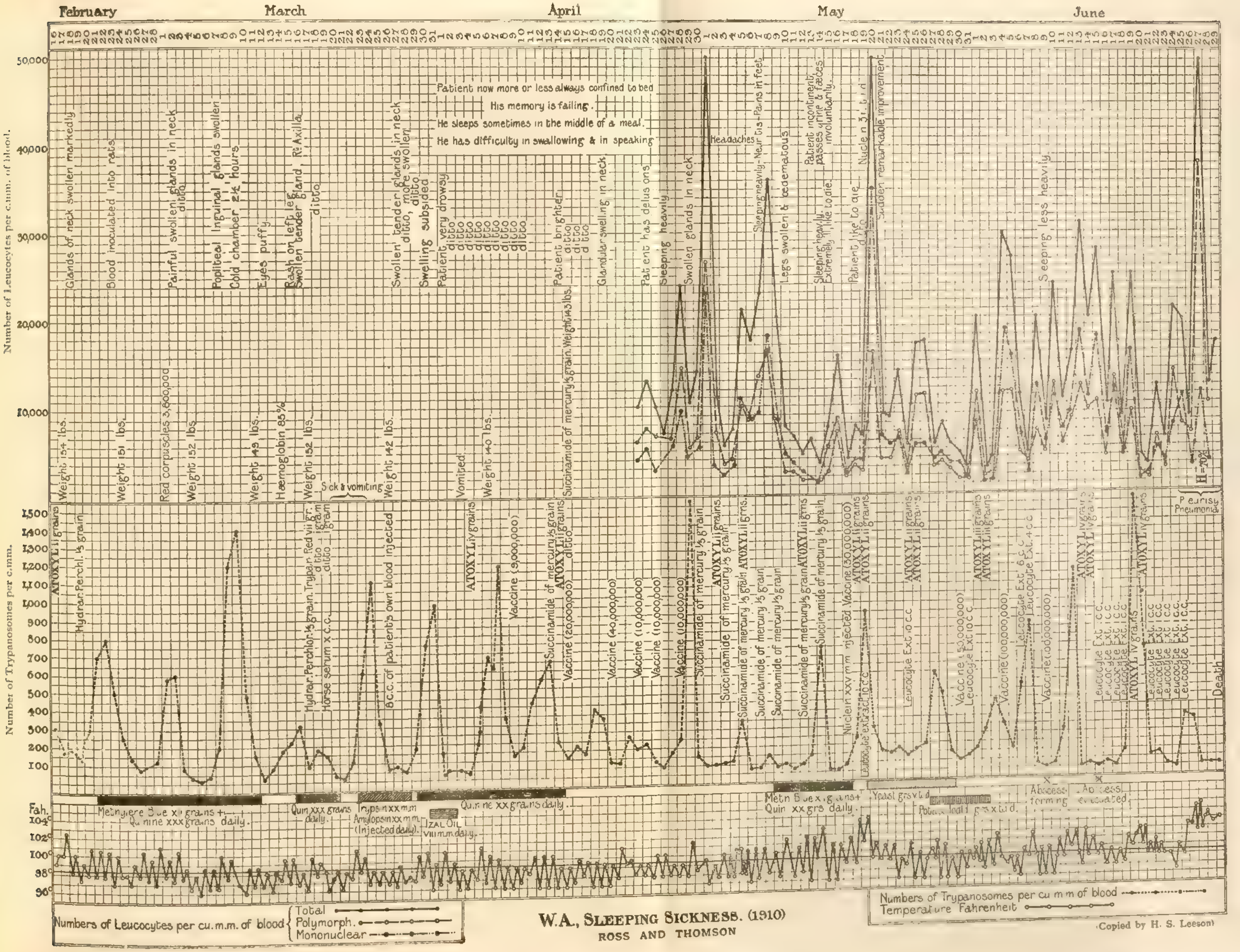
Date	12	13	14	15	16	17	18	19	20	21
Number of Tryps. per c.mm....	76	100	700	8	4	52	176	400	208	104
Maximum temp., H.F.	56	56	68	48	48	56	70	78	64	42
Average daily temp., H.F. ...	38	36	48	30	34	41	47	64	45	35
Total Leucocytes, in 100's ...	45	63	37	75	157	36	72	68	500	100
Polymorphs, in 100's	16	—	33	21	71	18	33	29	160	38
Mononuclears, in 100's	29	—	33	54	86	18	39	39	340	62
Mononuclears, per cent.	64	—	50	72	55	49	54	57	68	62
Hb. per cent. (Tallqvist)	80	75	80	80	80	80	80	80	80	85
Treatment	Succ. Hg, $\frac{1}{5}$ gr Atoxyl, 2 gr.	—	Succ. Hg. $\frac{1}{5}$ gr.	–		Vaccine, 30,000,000 Nuclein, 25 min.	Atoxyl. 2 gr.	Atoxyl, 2 gr. Leuc. ext.. 10 c.c.	—	

Date	May 22	May 23	May 24	May 25	May 26	May 27	May 28	May 29	May 30	May 31	June 1
Number of Tryps. per c.mm.	104	112	68	120	150	552	416	100	48	72	128
Maximum temp., H.F.	42	42	44	36	30	50	42	34	36	36	44
Average daily temp., H.F.	36	28	31	23	24	38	27	27	25	27	34
Total Leucocytes, in 100's	96	140	53	170	175	54	80	57	43	30	200
Polymorphs, in 100's	39	71	30	114	117	21	34	26	12	13	116
Mononuclears, in 100's	57	69	23	56	58	33	46	31	31	17	84
Mononuclears, per cent.	59	49	44	33	33	62	58	55	72	56	42
Hb. per cent. (Tallqvist)	85	—	85	85	85	85	85	85	80	80	80
Treatment			Atoxyl, 2 gr. Leuc. ext., 10 c.c.	Atoxyl, 2 gr.					Vaccine, 50,000,00	Leuc. ext., 10 c.c.	Atoxyl, 3 gr.

Date	June 2	June 3	June 4	June 5	June 6	June 7	June 8	June 9	June 10	June 11	June 12
Number of Tryps. per c.mm.	240	352	248	112	468	820	20	12	24	208	1164
Maximum temp., H.F.	44	56	54	30	38	56	40	40	46	56	58
Average daily temp., H.F.	34	33	36	21	24	39	27	26	30	41	48
Total Leucocytes, in 100's	28	40	300	270	110	45	200	80	240	108	160
Polymorphs, in 100's	14	23	114	119	45	27	72	33	130	49	74
Mononuclears, in 100's	14	17	186	151	65	18	128	47	110	59	86
Mononuclears, per cent.	50	43	62	56	59	40	64	59	46	55	54
Hb. per cent. (Tallqvist)	80	80	80	80	80	80	80	80	80	80	80
Treatment	Atoxyl, 3 gr.		Vaccine, 100,000,000		Leuc. ext., 4 c.c.	Leuc. ext., 4 c.c.		Vaccine, 100,000,000			

Date	13	14	15	16	17	18	19	20
	June							
Number of Tryps. per c.mm...	16	32	8	32	16	100	1452	680
Maximum temp., H.F.	60	58	50	38	38	50	50	60
Average daily temp., H.F. .	48	41	40	27	28	37	44	45
Total Leucocytes, in 100's ...	310	200	280	100	250	81	250	37
Polymorphs, in 100's	124	92	101	40	125	39	90	29
Mononuclears, in 100's	186	108	179	60	125	42	160	16
Mononuclears, per cent.	60	54	64	60	50	52	64	43
Hb. per cent. (Tallqvist)	80	80	-	-	80		80	80
Treatment	Atoxyl, 4 gr.	Atoxyl, 4 gr.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Atoxyl, 4 gr.	Atoxyl, 4 gr.

Date	21	22	23	24	25	26	27	28	29
	June								
Number of Tryps. per c.mm....	56	72	40	24	300	260	40	40	30
Maximum temp., H.F.	42	42	34	40	66	88	78	74	70
Average daily temp., H.F. ..	35	34	30	27	42	75	70	69	70
Total Leucoctes in 100's	32	120	60	210	195	84	500	126	—
Polymorphs, in 100's	13	61	28	147	78	66	380	105	—
Mononuclears, in 100's	19	59	32	63	117	18	120	21	—
Mononuclears, per cent.	60	49	54	35	60	21	24	17	—
Hb. per cent. (Tallqvist)	80	70	70	70	75	70	70	70	—
Treatment	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.				—



W.A., Sleeping Sickness. (1910)

ROSS AND THOMSON

(Copied by H. S. Leeson)

ENUMERATIVE STUDIES ON *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* IN RATS, GUINEA-PIGS, AND RABBITS; PERIODIC VARIATIONS DISCLOSED*

BY
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(Received for publication 15 *October*, 1910)

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INTRODUCTION

The results recorded in this memoir were obtained chiefly from investigations on animals—rats, guinea-pigs and rabbits—sub-inoculated from a case of Rhodesian sleeping sickness recently studied by Major R. Ross and Dr. D. Thomson in the Royal Southern Hospital, Liverpool.

The investigations were undertaken at the suggestion of Major Ross, under funds allotted by the Tropical Diseases Research

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* An abstract of this paper was read before the Royal Society on December 8, 1910, and published in Proc. Roy. Soc., B. Vol. LXXXIII, pp. 206-211.

Committee in the case of one of us (H.B.F.), and under the Sir Edwin Durning-Lawrence Fund in the case of the other (J.G.T.). The researches on animals were necessary in order to determine whether a regular periodic increase in the parasites in the peripheral circulation occurred, similar to that found by R. Ross and D. Thomson* (1910) in the case of Rhodesian sleeping sickness. Again, it was necessary to determine whether this numerical cyclical development had a definite significance in the life-history of the parasite, which it would be necessary to consider in the case of treatment of trypanosomiasis by drugs or other methods. Our work was done in the Liverpool School of Tropical Medicine.

The trypanosome from the patient suffering from sleeping sickness contracted in Rhodesia was found by Stephens and Fantham (1910)† to exhibit a marked morphological peculiarity in the presence of a posterior nucleus in some of the stout forms, and for this parasite the name *T. rhodesiense* was suggested by them. We have also used an old laboratory strain of *T. gambiense*.

OUTLINE OF THE METHODS USED

In all cases a definite number of trypanosomes was inoculated. The inoculation was made subcutaneously in rats, intraperitoneally in guinea-pigs and rabbits.

The peripheral blood of these animals was then examined daily, at the same hour, that is to say, at regular intervals of twenty-four hours.

The technique employed was that elaborated by R. Ross and D. Thomson (1910) in their study of the patient W.A., namely, that a known quantity of blood (one cubic millimetre divided into quarters) was taken and the parasites therein very carefully counted. In counting the thick films an Ehrlich eye-piece was used, after the films had been dehaemoglobinised, fixed with absolute alcohol and stained by the Romanowsky method.

When the parasites became very numerous it was necessary to spread the quarter cubic millimetre of blood over a larger square area than usual, and to use a very small diaphragm in the eye-piece. The parasites were then counted in three or four bands across the film, which had been previously examined in order to ascertain an even distribution of the trypanosomes therein—though this led to certain unavoidable errors (see R. Ross and D. Thomson on 'Enumerative Studies on Malarial Fever'*). It will be obvious that the counts entailed much labour, especially when the parasites were numerous.

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* Proc. Roy. Soc., B, Vol. LXXXII. pp. 411-415, and Vol. LXXXIII. pp. 187-205.
† Proc. Roy. Soc., B. Vol. LXXXIII. pp. 28-33.

We have endeavoured to estimate the number of divisions of the trypanosomes during the first twenty-four hours of their appearance in the peripheral blood of the host. (See pp. 433-4.)

ENUMERATIONS IN RATS

We now give a series of tables of enumerations in rats inoculated with (*a*) *T. rhodesiense* and (*b*) *T. gambiense*.

In the case of both strains it was found that the rats tended to fall into one of two main categories exhibiting either (1) a periodic increase and decrease in the number of parasites in the peripheral blood (see Chart 1), or (2) a more or less continuous rise in the numbers of parasites until the animal died (see Chart 2). (Cf. Moore and Breinl†.)

We therefore classify the animals inoculated with each strain into two groups:—

(*a*) *Trypanosoma rhodesiense*

I. RATS EXHIBITING PERIODIC VARIATION

RAT I.—Piebald ♂, weight 187 grams. Dose of inoculation one million Trypanosomes. (H.B.F.)

Days	1	2	3	4	5	6	7
Parasites per c.mm.	–	–	6	48	9,860	60,000	84,640
Temp.[1]	30	32	44	64	64	50	50

Day	8	9	10	11	12	13	14
Parasites per c.mm.	7,760	24,000	20,480	63,800	32,000	21,760	89,600
Temp.[1] . ..	28	10	2	30	22	0	10

[1] The temperature is expressed according to the following Haematothermic scale, as in Major Ross's recent papers on 'Malaria' and 'Trypanosomiasis':—Temp. = (F − 95) × 10, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer, per rectum.

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* Proc. Roy. Soc., B, Vol. LXXXIII, p. 161, and Ann. Trop. Med. and Parasitol. (1910), Vol. IV, p. 268.

† Ann. Trop. Med. and Parasitology (1907), Vol. I, p. 448.

The graph of the number of parasites of this animal is represented in Chart 1.

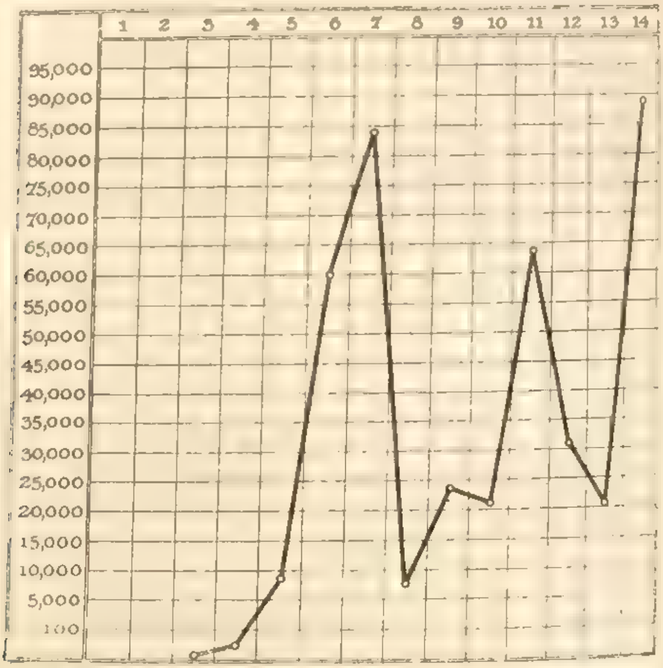

CHART 1.—Graph showing Daily Counts of the Parasites per c.mm. in the Peripheral Blood of a Rat inoculated with *T. rhodesiense.* Periodic Variation.

The numbers of the parasites are indicated along the vertical line, and the duration of infection in days along the horizontal line.

(See record of Rat 1.)

RAT 2.—White ♂, weight 108 grams. Dose 350,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	68	18,240	44,120	32,080	22,40
Temp.	32	24	20	40	56	38	22

Day	8	9	10	—	—	—	—
Parasites per c.mm.	12,860	126,000	61,840	—	—	—	—
Temp.	20	25	4	—	—	—	—

RAT 3.—White ♀, weight 225 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	12	3,480	58,240	16,000	1,840	6,400
Temp.	20	20	14	86	106	40	15
Day	8	9	—	—	—	—	—
Parasites per c.mm.	52,000	42,400	—	—	—	—	—
Temp.	15	0	—	—	—	—	—

RAT 4.—Piebald ♂, weight 220 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	8	1,960	11,520	28,800
Temp.	40	53	50	50	34	36	74
Day	8	9	10	11	12	13	—
Parasites per c.mm.	56,320	84,000	50,000	39,200	160,000	100,400	—
Temp.	50	48	38	40	47	20	—

RAT 5.—White ♀, weight 120 grams. Dose: 750,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	896	3,280	32,560	51,600
Temp.	38	36	38	50	40	52	48
Day	8	9	10	11	—	—	—
Parasites per c.mm.	64,800	22,800	16,800	6,160	—	—	—
Temp.	44	22	20	10	—	—	—

RAT 6.—Piebald ♂, weight 100 grams. Dose: one million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	32	8,400	52,728	67,760
Temp.	40	40	46	56	60	50	[illegible]
Day	8	9	—	—	—	—	—
Parasites per c.mm.	96,000	40,656	—	—	—	—	—
Temp.	30	54	—	—	—	—	—

RAT 7.—Piebald ♂, weight 189 grams. Dose: 30,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	[illegible]
Parasites per c.mm.	—	—	336	6,572	23,888	80,640	40,60[illegible]
Temp.	38	40	70	72	66	70	44
Day	8	9	—	—	—	—	—
Parasites per c.mm.	30,250	150,000	—	—	—	—	—
Temp.	42	5	—	—	—	—	—

RAT 8.—White ♀, weight 195 grams. Dose: 100,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	100	0	896	60,000
Temp.	40	42	40	40	60	64	74
Day	[illegible]	9	10	11	12	13	—
Parasites per c.mm. ...	81,000	9,200	1,960	7,840	23,040	83,200	—
Temp.	55	56	70	60	68	52	—

RAT 9.—Piebald ♂, weight 100 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	20	4,672	8,040	70,400
Temp.				30	40	50	54

Day	8	9	10	11	12	—	—
Parasites per c.mm.	32,400	3,360	29,880	88,800	113,520	—	—
Temp.	58	46	42	40	35		

RAT 10.—Piebald ♂, weight 150 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	12	3,620	8,400	2,520	7,280
Temp.	36	34	42	50	42	66	48	38

Day	9	10	11	12	13	14	15
Parasites per c.mm.	12,000	6,200	7,040	11,000	80,000	18,432	120,000
Temp.	46	34	22	40	44	24	20

Note slow rise in numbers of parasites.

RAT 11.—White ♀, weight 148 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	316	1,920	8,800	11,200
Temp.						54	66

Rat 11—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	2,580	1,040	52	1,032	2,348	26,400	54,00
Temp.	66	52	50				

Day	15	16	17	18	—	—	—
Parasites per c.mm.	7,360	12,120	31,460	85,460	—	—	—
Temp.					—	—	—

Note the slow rise, and the long period between the crests of the first waves, due probabl to the great resistance of the animal.

Rat 12.—White ♀, weight 138 grams. Dose: 600,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	16	3,620	13,740
Temp.	34	42	54	44	54	74	66

Day	8	9	10	11	12	—	—
Parasites per c.mm.	34,272	6,048	3,640	24,360	21,600	—	—
Temp.	64	60	40	63	46	—	—

This animal was pregnant, and gave birth to five young rats on the tenth day.

Rat 13.—White, weight 140 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	84	8,928

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	0	0	560	0	7,004	1,408	2,856

Rat 13—continued.

Day	15	16	—	—	—	—	—
Number of Trypanosomes per c mm.	118,272	162,288	—	—	—	—	

This animal was pregnant, and gave birth to eight young rats on ninth day.

Rat 14.—Piebald, weight 127 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c mm.	—	—	—	1,440	45,600	66,240	7,144

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	50,232	77,440	250,000	464,640	500,000	—	

Rat 15.—Piebald, weight 104 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	4,620	45,440

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	24,472	44,352	194,128	410,000	—	—	

Rat 16.—White, weight 58 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	104	37,000	30,240
Temp.		42	44	36		44	46

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	—	—
Temp.	34	52	20	34	18	—	—

Note 'staircase' rise in the numbers of the parasites.

Rat 17.—White, weight 62 grams. Dose of inoculation = 38,000 Trypanosomes. (J.G.T.

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—		—	1,500	500	1,380	4,02
Temp.		50	30	56	50			70

Note initial fall in numbers during first 24 hours.

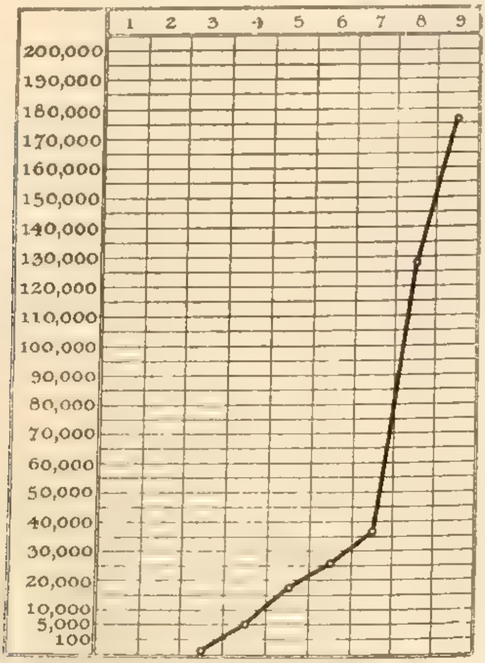

Chart 2. Graph of Daily Counts of the Parasites in the Blood of a Rat inoculated with *T. rhodesiense.* Continuous rise. (See record of Rat 18.)

II.—RATS EXHIBITING CONTINUOUS RISE IN THE NUMBER OF PARASITES

Rat 18.—White ♂, weight 108 grams. Dose of inoculation: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	20	4,888	17,940	26,240	36,000
Temp.			34	42	50	48	30

Rat 18—continued.

Day	8	9	—	—	—	—	—
Parasites per c.mm.	129,024	175,200	—	—	—	—	—
Temp.		46	—	—	—	—	—

The graph of Rat 18 is represented in Chart 2.

Rat 19.—Piebald, weight 137 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	–	—	—	48	4,920	9,800	26,880
Temp.	40	40	52	62	50	62	52

Day	[illegible]	9	10	—	—	—	—
Parasites per c.mm.	31,000	51,080	160,000	—	—	—	—
Temp.	44	44	30	—	—	—	—

Rat 20.—Piebald ♂, weight 182 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	[illegible]
Parasites per c.mm.	—	—	20	4,352	6,640	7,776	20,088	126,000
Temp.	36	36	34	52	48	47	10	0

Rat 21.—Piebald ♂, weight 130 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	36	3,888	42,200	58,800
Temp.	38	36	38	50	34	58	20

Day	8	9	10	11	—	—	—
Parasites per c.mm.	86,832	97,200	112,000	144,000	—	—	-
Temp.	16	50	20	0	—	—	—

Rat 22.—White ♀, weight 125 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.,

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	244	18,000	66.560	81,000	176.8
Temp.	20	22	30	26	20	6	

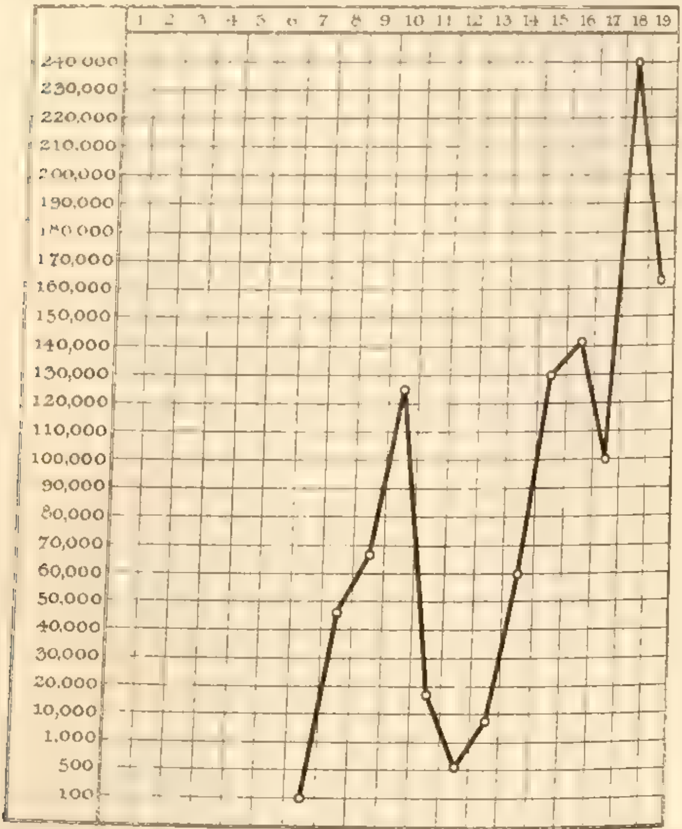

Chart 3.—Graph showing Daily Counts of the Parasites per c.mm. in the Peripheral Blood of a Rat inoculated with *T. gambiense*. Periodic Variation. (See record of Rat 23.)

(*b*) *Trypanosoma gambiense*

I.—RATS EXHIBITING PERIODIC VARIATION

Rat 23.—Piebald, weight 173 grams. Dose of inoculation . two million Trypanosomes (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	12
Temp.	55	40	50	50	44	34	51

Rat 23—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	48,000	68,728	125,860	18,764	600	9,280	60,712
Temp.	45	65	50		50	66	38

Day	15	16	17	18	19	—	—
Parasites per c.mm.	130,000	142,000	100,000	240,000	164,000		—
Temp.	34	40	55		11		—

The graph of Rat 23 is represented in Chart 3.

RAT 24.—White ♂, weight 165 grams. Dose of inoculation: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	-		4	6,000	1,920
Temp.		30	34	34	60	64	74	52

Day	9	10	11	12	13	14	15
Parasites per c.mm.	120	200	300	360	28,800	16,800	138,240
Temp.	54		20	54	54	58	10

RAT 25.—Piebald ♂, weight 155 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.			-	3,080	17,080	90,000	160,000
Temp.		50	22	10	44		66

Day	8	9	10	11	12	13	—
Parasites per c.mm.	19,100	7,800	7,800	10,080	76,800	38,800	—
Temp.	20	42	40	10	0	0	—

Rat 26.—White ♀, weight 150 grams. Dose: 150,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	—	4	1,068
Temp.	48	55	46	65	60	62	58	5

Day	9	10	11	12	13	14	15
Parasites per c.mm.	4,892	768	1,680	2,860	9,688	6,384	2,436
Temp.	61	60	44	46	57	54	60

Rat 27. White, weight 113 grams. Dose of inoculation: 60,000 Trypanosomes. J.G.T

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	120	14,310	101,740
Temp.	61	49	32	12	44		5	40

Day	9	10	11	12	13	14	15
Parasites per c.mm.	146,880	69,400	23,276	55,224	67,096	57,552	6,120
Temp.	44	36	36	35			

Rat 28.—White, weight 101 grams. Dose of inoculation: 60,000 Trypanosomes. J.G.T

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	-	—	—	—	256	2,80
Temp.	59	70	43	42	40		56

Day	8	9	10	11	12	13	14
Parasites per c.mm.	4,416	3,648	24,904	90,560	118,700	143,520	270,00
Temp.	44	42	34	26	24		40

Rat 28—continued.

Day	15	16	17	—	—	—	—
Parasites per c.mm.	100,000	200,600	6,048	—	—	—	—
Temp.	78	42	—	—	—	—	—

Rat 29.—Piebald ♂, weight 230 grams. Dose: one million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.		—	364	3,328	6,600	28,080	12,600
Temp.	36	40	56	60	47	82	56

Day	8	9	10	11	—	—	—
Parasites per c.mm.	2,808	16,380	19,360	150,000	—	—	—
Temp.	54	52	44	10	—	—	—

II.—RATS EXHIBITING CONTINUOUS RISE IN THE NUMBER OF PARASITES

Rat 30.—Piebald ♀, weight 125 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.			—	—	—		
Temp.	46	76	72		56	50	48

Day	8	9	10	11	12	13	14
Parasites per c.mm.		8	2,112	2,600	3,120	150,000	153,000
Temp.	62	65	42		34	58	26

Day	15	16	—	—	—	—	—
Parasites per c.mm.	181,440	540,000	—	—	—	—	—
Temp.	36	10	—	—	—	—	—

RAT 31.—Piebald ♂, weight 100 grams. Dose: 85,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	3,000	12,000	15,12
Temp.		34		30	34	32	4

Day	8	9	10	11	12	—	—
Parasites per c.mm.	24,000	101,400	200,600	378,560	216,000	—	—
Temp.	34	44	42	20	10	—	—

RAT 32.—Piebald ♀, weight 155 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	1,680	14,560	30,200	61,250	90,000
Temp.	34		34	66	52	50	44

Day	8	9	10	11	—	—	—
Parasites per c.mm. . ..	220,500	409,600	504,000	360,000	—	—	—
Temp.	50	44	34	10	—	—	—

RAT 33.—White ♀, weight 40 grams. Dose: 100,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.		—	32	8,100	48,150	88,000	126,000	180,000
Temp.			34		45		30	7

This rat was young. It is interesting to note that a count was made on the third day, five hours after the first count recorded in the table. There were then 544 parasites per c.mm., or $\frac{544}{32} = 17$ times the first count, showing that four divisions of the parasite had taken place in five hours.

From these tables it will be seen that young and light rats, such as Numbers 18 and 33, are less resistant than older and heavier rats, e.g., Numbers 1 and 23.

It will also be seen that when the initial rise is rapid and high the rat soon dies, and does not show periodic variation.

COMPARISON OF THE TWO STRAINS IN RATS

We now append a table, for purposes of easy comparison, of various points regarding the two strains in rats. The figures are compiled from the foregoing data, and relate to enumerations in seventeen rats exhibiting periodic variation, and of five rats exhibiting continuous rise in the case of *T. rhodesiense*; also of seven rats exhibiting periodic variation and of four rats showing continuous rise in the case of *T. gambiense*.

(*a*) *T. rhodesiense*

No.	Colour	Weight in grams	Incubation period in days	Duration of life in days	No. of Trypanosomes inoculated	Approximate No. of Divisions in first 24 hours	Remarks
1	Piebald	187	2	14	1 million	3	(7 to 8 divisions in next 24 hours)
2	White	108	2	10	350,000	7	
3	White	225	1	9	2 million	8	
4	Piebald	220	3	13	500,000	8	
5	White	120	3	11	750,000	2	
6	Piebald	100	3	9	1 million	8	
7	Piebald	189	2	9	30,000	4	
8	White	195	3	13	100,000	Fall	(10 divisions in next 24 hours)
9	Piebald	100	3	12	500,000	8	
10	Piebald	150	3	15	500,000	8	
11	White	148	3	18	500,000	3	
12	White	138	4	12	600,000	8	Pregnant
13	White	140	4	16	200,000	4	Pregnant
14	Piebald	127	3	12	200,000	5	
15	Piebald	104	4	11	200,000	10	
16	White	58	4	12	200,000	8	
17	White	62	4	8	38,000	Fall	
18	White	108	2	9	2 million	8	See Chart 2
19	Piebald	137	3	10	1½ million	7	
20	Piebald	182	2	8	2 million	8	
21	Piebald	130	3	11	1½ million	7	
22	White	125	2	7	1½ million	6	
				Average ...	780,000		

(β) *T. gambiense*

No.	Colour	Weight in grams	Incubation period in days	Duration of life in days	No. of Trypanosomes inoculated	Approximate No. of Divisions in first 24 hours	Remark
23	Piebald	173	6	17	2 million	8	See Chart
24	White	165	5	15	500,000	10	
25	Piebald	155	3	13	2 million	3	
26	White	150	6	15	150,000	8	
27	White	113	5	15	60,000	7	
28	White	101	5	17	60,000	3	
29	Piebald	230	2	11	1 million	3	
30	Piebald	125	8	16	500,000	8	
31	Piebald	100	4	12	85,000	2	
32	Piebald	155	2	11	1,500,000	3	Not very resist
33	White	40	2	8	100,000	8	Young
				Average ...	723,000		

From the foregoing tables it follows:—

(1) That the average incubation period* in the case of *T. rhodesiense* was 2·9 days, whereas the average incubation period in the case of *T. gambiense* was 4·4 days.

(2) The average life of rats inoculated with *T. rhodesiense* was 11·3 days, whereas the life of rats infected with *T. gambiense* was 13·8 days.

These inferences are legitimate, for the average weight of the rats inoculated with *T. rhodesiense* was 138·8 grams, whereas the average weight of those inoculated with *T. gambiense* was 13 grams. Also the numbers of trypanosomes inoculated were nearly the same in the two strains, averaging 780,000 and 723,00 respectively.

(3) On comparing the number of divisions of parasites occurring in the first twenty-four hours in both strains, we find that it was approximately the same, namely, six divisions on the average, and varied in each case from two to ten divisions, most frequently being eight.

(4) There is also a shorter period between the crests of the waves in the graphs denoting the number of trypanosomes in

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[*] By incubation period we mean the interval (in days) from the moment of inoculation of the parasites into the host till the time the trypanosomes are first found in one cubic millimetre of the peripheral blood of host.

the Rhodesian strain (about three to four days) than in the laboratory strain of *T. gambiense* (viz., four to six days). (See Charts 1 and 3.)

(5) The difference in morphology between *T. rhodesiense* and *T. gambiense* has already been noted by Stephens and Fantham (1910).

(6) It follows, especially from the incubation period and the duration of life in sub-inoculated rats, that the Rhodesian strain is the more virulent. The difference of virulence is more markedly shown in the duration of life of infected guinea-pigs (see p. 457).

Curiously enough, we find that the incubation period does not seem to be affected so much by the dose (number of trypanosomes) inoculated as by the resistance of the animals. (Cf. Professor Ross's 'Prevention of Malaria' (1910), pp. 94, 95.)

ENUMERATIONS IN GUINEA-PIGS

Exactly the same methods as already set forth were followed in the counts of parasites in guinea-pigs. The same two strains of trypanosomes were again used, five animals being inoculated with each strain. We append tables of our results, together with typical charts.

1. *T. rhodesiense*

Guinea-pig 1.—Weight 346 grams. Dose of inoculation: 500,000 Trypanosomes. Incubation period 6 days. Duration of life 79 days. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.		–		–	–	—	4
Leucocytes	–	6,000	7,680		13,000	6,720	3,884
Temp.	–	80	84	76	–	70	76
Weight in grams		346	—	—	–	—	

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	28	44	20	8	48	2	4
Leucocytes	18,944	11,328	13,440	4,920	26,400	10,780	9,408
Temp	75	74	70	78	—	74	80
Weight in grams		–	—	–	—	—	—

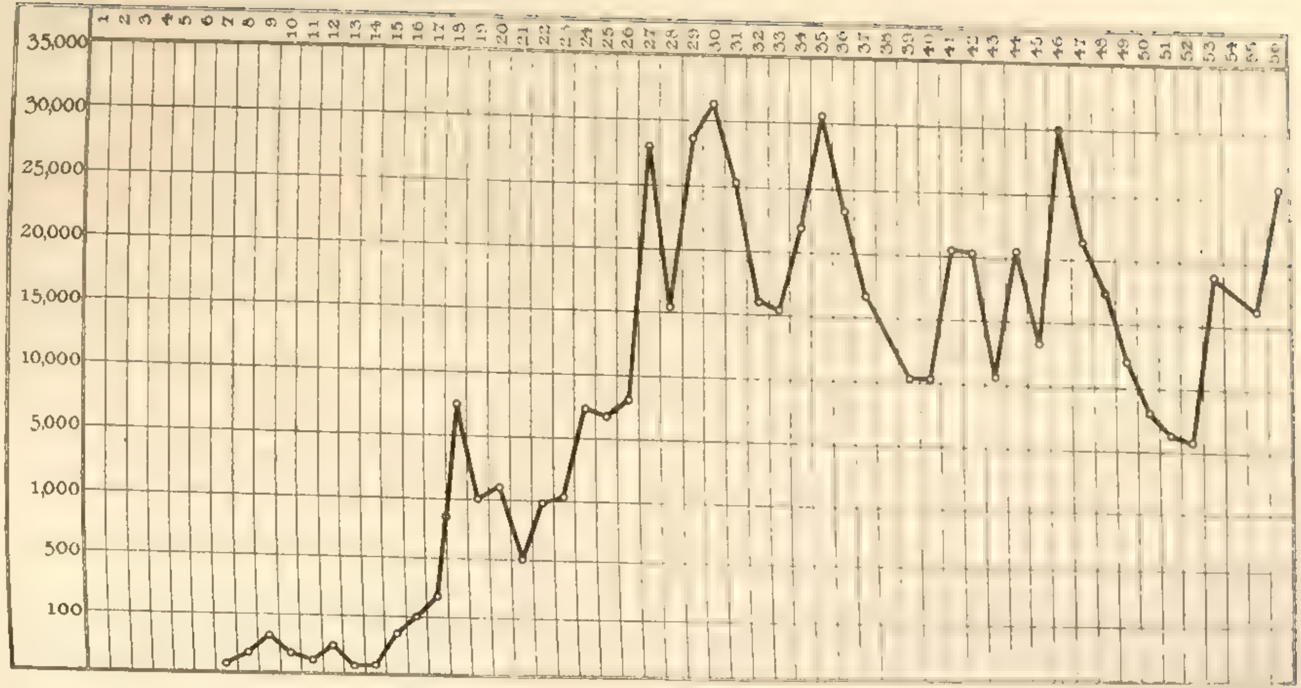

CHART 4.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation. (See record of Guinea-pig 1.)

Guinea-pig 1—continued.

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	68	104	216	8,712	1,010	1,936	504
Leucocytes	—	—	—	—	—	—	—
Temp.	80	80	80	66	—	76	80
Weight in grams		408				-	

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	1,152	1,360	8,576	8,064	9,936	28,224	15,504
Leucocytes		4,200	24,384	14,400	24,360		
Temp.	76	76	74	76		80	81
Weight in grams		—	—	—	—	—	-

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	29,488	32,320	25,200	16,800	15,792	23,200	30,944
Leucocytes	—	—	—	—	—	—	
Temp. ...	80	82	80	80	—		94
Weight in grams		425	—	479	—	—	

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	23,688	16,800	—	10,560	10,520	21,280	20,208
Leucocytes	—	—	—	—	—	—	—
Temp.	81	83	—	—	—	82	
Weight in grams	—	—	—	—	—	-	—

Day .. .	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	11,172	21,334	14,784	30,448	24,812	17,952	12,960
Leucocytes		—	—	—	—	—	·
Temp.			—	—	—	·	—
Weight in grams				—		—	—

Guinea-pig 1—continued.

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	9,920	8,920	7,448	19,824	–	17,612	26,496
Leucocytes	—		—	–	—	—	·
Temp.		—	–	-	—	—	—
Weight in grams ..	—	—	—	—	—	–	-

The animal lived for 23 days after the last count was made.

The graph of Guinea-pig 1 is shown in Chart 4.

Guinea-pig 2, ♂.—Weight 880 grams when inoculated; 614 grams at death. Dose of inoculation: 500,000 Trypanosomes. Duration of life 28 days. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	-	2	0	1	4	4	12
Temp.		12	70	66	26		65	62

Day	9	10	11	12	13	14	15
Parasites per c.mm.	8	100	150	1,000	896	1,680	7,992
Temp.	74	70	64	66		76	68

Day	16	17	18	19	20	21	22
Parasites per c.mm.	6,000	5,632	7,200	21,600	45,600	25,000	11,340
Temp.	63	75	80	70	73	52	20

Day	23	24	25	26	27	28	
Parasites per c.mm.	6,720	4,416	18,720	9,600	6,000	30,720	Died
Temp.	80	82	10	64	78	6	

Guinea-pig 3, ♂.—Weight 952 grams at time of inoculation; 880 grams at death. Dose of inoculation: two million Trypanosomes. Duration of life 82 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	-	-	—	—	-	8	4
Temp.		25				20	
Day			46	47	48	49	50
Parasites per c.mm.	Interval of 38 days' vacation		12,600	80,064	68,448	23,562	9,972
Temp.			32	44	40	38	34
Day	51	52	53	54	55	56	57
Parasites per c.mm.	4,840	2,320	6,040	7,200	3,600	2,560	4,284
Temp.		44	30	40	68	26	28
Day ..	58	59	60	61	62	63	64
Parasites per c.mm.	8,600	12,480	23,296	3,328	4,320	1,600	3,072
Temp.		40	40	41	34	30	34
Day ...	65	66	67	68	69	70	71
Parasites per c.mm.	6,400	8,640	1,280	2,080	1,024	768	1,2[illegible]
Temp.		46	30	44	40	42	43
Day	72	73	74	75	76	77	78
Parasites per c.mm.	10,560	6,048	5,880	2,480	4,320	5,760	4,032
Temp.		38	40	32	32	33	26

Guinea-pig 3—continued.

Day	79	80	81	82			—
Parasites per c.mm.	1,296	720	140	6,000	Died	—	—
Temp.	24	4	36	14			

GUINEA-PIG 4, ♂.—Weight 440 grams at time of inoculation; 402 grams at time of death. Dose of inoculation: two million Trypanosomes. Duration of life 57 days. (H.B.F.)

Day	1	2	3	4	5	6	7	[illegible]
Parasites per c.mm.					1,920	8	8	[illegible]
Temp.				13	22	31	22	[illegible]

Day		47	48	49	50	51
Parasites per c.mm. ..	Interval of 38 days' vacation	10,000	67,392	113,568	33,880	96,10[illegible]
Temp		20	24	46	32	

Day	52	53	54	55	56	57	
Parasites per c.mm.	50,000	26,740	14,864	7,168	3,024	24,720	Died
Temp.	40	36	36	49	34	2	

GUINEA-PIG 5, ♀.—Weight 492 grams when inoculated; 447 grams at death. Dose of inoculation: 1½ million Trypanosomes. Duration of life 51 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm. . .			—	—	—	—	—
Temp.			30				

Day	8	9	10	11	12	13	14
Parasites per c mm.	—	—		12	4	56	40
Temp.	44	40	48	48	46	40	

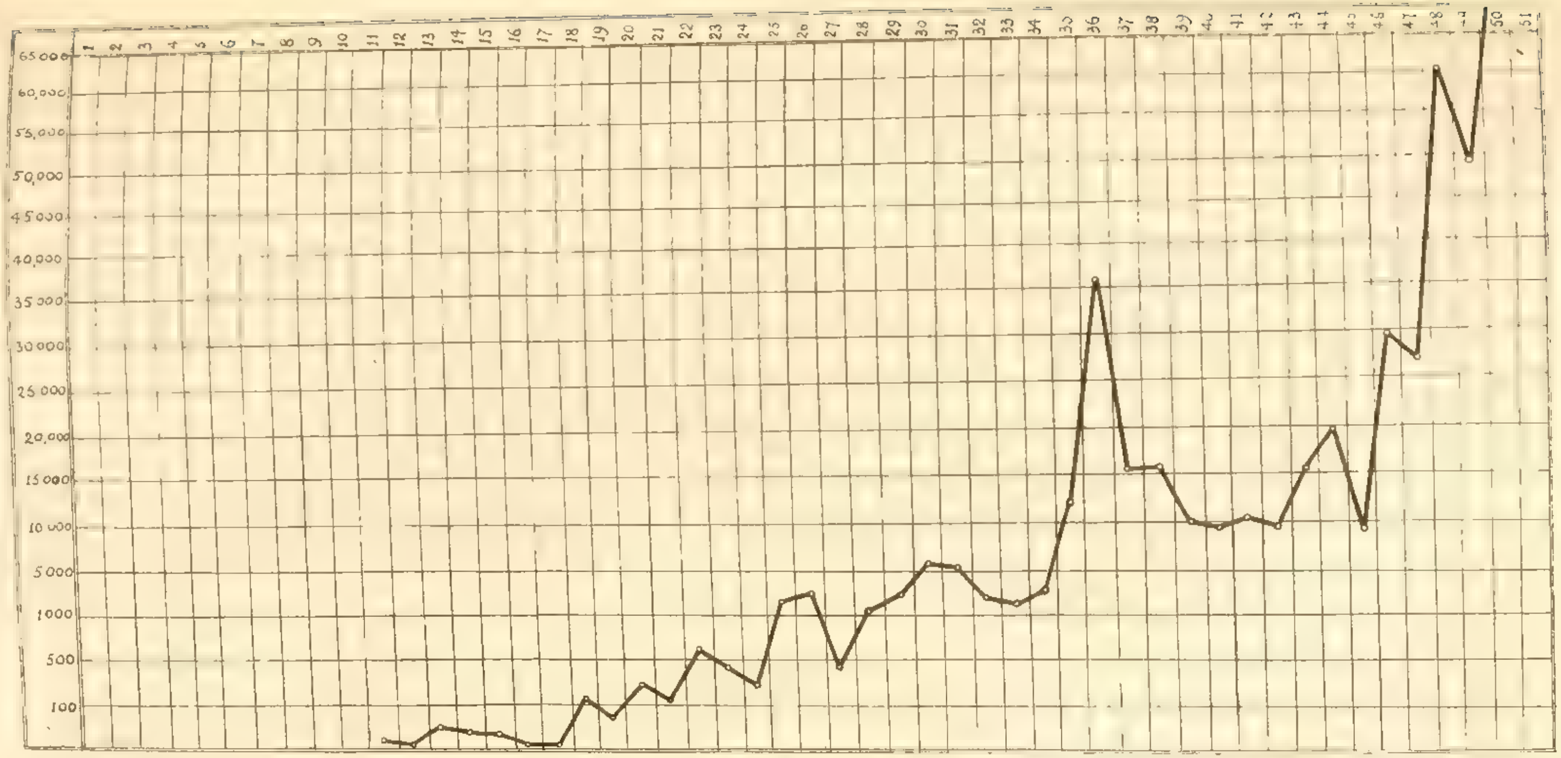

CHART 5.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation. (See record of Guinea-pig 5.)

Guinea-pig 5—continued.

Day	15	16	17	18	19	20	21
Parasites per c.mm. ...	36	4	4	148	76	240	12
Temp.	36	52	30	46	34	30	24
Day	22	23	24	25	26	27	28
Parasites per c.mm. ...	660	42	260	2,700	3,264	432	1,024
Temp.	40	26	12	12	25	26	
Day	29	30	31	32	33	34	35
Parasites per c.mm. ...	3,200	6,400	5,440	2,744	2,352	3,520	12,8
Temp.	23	38	24	38	45	20	
Day	36	37	38	39	40	41	42
Parasites per c.mm.	35,200	15,680	15,760	10,000	9,408	10,240	9,80
Temp.	50	47	38	40	40	36	
Day	43	44	45	46	47	48	49
Parasites per c.mm.	15,120	19,600	9,600	29,568	27,320	60,624	49,140
Temp.	50	44	23	30	28	54	50
Day	50	51					
Parasites per c.mm.	96,880	164,160	Died		–	–	-
Temp.	38	-				-	-

The graph of Guinea-pig 5 is represented in Chart 5.

2. *T. gambiense*

Guinea-pig 6.—Weight 403 grams. Dose of inoculation: 4,000,000 Trypanosomes. Duration of life 79 days. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	12	16	24	8	0
Leucocytes	—	—	—	—	-		—
Temp.	78	73	91	82	84	70	77
Weight in grams	403						

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c mm.	8	8	4	0	8	76	48
Leucocytes	—	—	—	—	—	—	—
Temp.	76	85	86	—	90	80	91
Weight in grams	-	-	—	—	—	-	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	680	1,440	960	—	272	440	416
Leucocytes	-		-	—	—	—	—
Temp.	80	76	80	—	74	76	88
Weight in grams	405	—	—	—	—	—	-

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	1,296	420	480	270	2,552	1,320	6,000
Leucocytes	—	—	—	-	—	—	—
Temp.	81	90	82	-	—	72	80
Weight in grams	—	-	—	—	—	—	—

Guinea-pig 6—continued.

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	13,392	21,620	29,160	44,016	28,836	14,812	[illegible]
Leucocytes	—	—	13,428	-	16,200	7,452	[illegible]
Temp.	74		86	-	84	80	[illegible]
Weight in grams	427			—	—	—	-

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	29,592	39,720	52,600	39,396	27,840	67,032	[illegible]
Leucocytes	8,856	11,200	12,400	5,964	—	7,068	-
Temp.	70	76	70		82	76	82
Weight in grams . .	—			—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	88,000	100,400	45,080	110,400	60,904	40,240	55,240
Leucocytes	6,920	8,320	5,100	14,812	6,888	6,240	[illegible]
Temp.	82	82	80	—	70	78	[illegible]
Weight in grams	538	—	—	—	—	-	—

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	32,544	99,000	71,680	75,674	74,784	74,112	[illegible]
Leucocytes	4,032	8,530			—	-	-
Temp.	81	84	86		86	78	78
Weight in grams	—	.	--	—	—	—	—

Guinea-pig 6—continued.

Day	57	58	59	60	61	62	63
Number of Trypanosomes per c.mm.	67,926	85,184	26,460	56,280	33,600	70,752	80,726
Leucocytes			9,976				
Temp.	76	76	78	—	-	99	80
Weight in grams	574					—	

Day	64	65	66	67	68	69	70
Number of Trypanosomes per c.mm.	64,000	50,232	27,456	44,000	63,962	91,056	72,432
Leucocytes	—					19,152	
Temp.	80		—	—	76	—	—
Weight in grams	610	—				-	—

Day	71	72	73	74	75	76	77
Number of Trypanosomes per c.mm.	57,624	62,800	72,384	100,128	62,496	75,520	49,856
Leucocytes				—		—	—
Temp.					—	—	—
Weight in grams			-				

Day	78	79	-	—	—		
Number of Trypanosomes per c.mm.	80,808	53,176	Died	-	—	—	—
Leucocytes	—				-	-	-
Temp.			—	—	--	-	—
Weight in grams			-		—	—	—

Guinea-pig 7.—Incubation period 4 days. Duration of life 105 days. (J.G.T.)

Day	20	21	22	23	24	25	26
Number of Trypanosomes per c.mm.	30,190	15,120	6,784	4,280	3,852	30,400	42,008
Temp.	—	86	80	76	83	89	[illegible]
Weight in grams	—	—	—	534	—	—	—
Leucocytes		—	—		—	-	12

Day	27	28	29	30	31	32	33
Number of Trypanosomes per c.mm.	32,212	28,120	7,914	18,698	13,860	23,440	21,720
Temp.	86	80	85	84	82	85	-
Weight in grams	—	—		—			-
Leucocytes	—					—	-

Day	34	35	36	37	38	39	40
Number of Trypanosomes per c.mm.	8,040	13,784	8,460	9,720	9,600	32,572	-
Temp.	84	107	90	80	76	78	-
Weight in grams	-	—	—	467	—	-	—
Leucocytes			—			-	—

Day	41	42	43	44	45	46	47
Number of Trypanosomes per c.mm.	16,992	10,000	3,840	432	1,760	1,940	32
Temp.	82	80	82	84	84	86	-
Weight in grams	—	—			—	-	—
Leucocytes	-	—	—	—		-	-

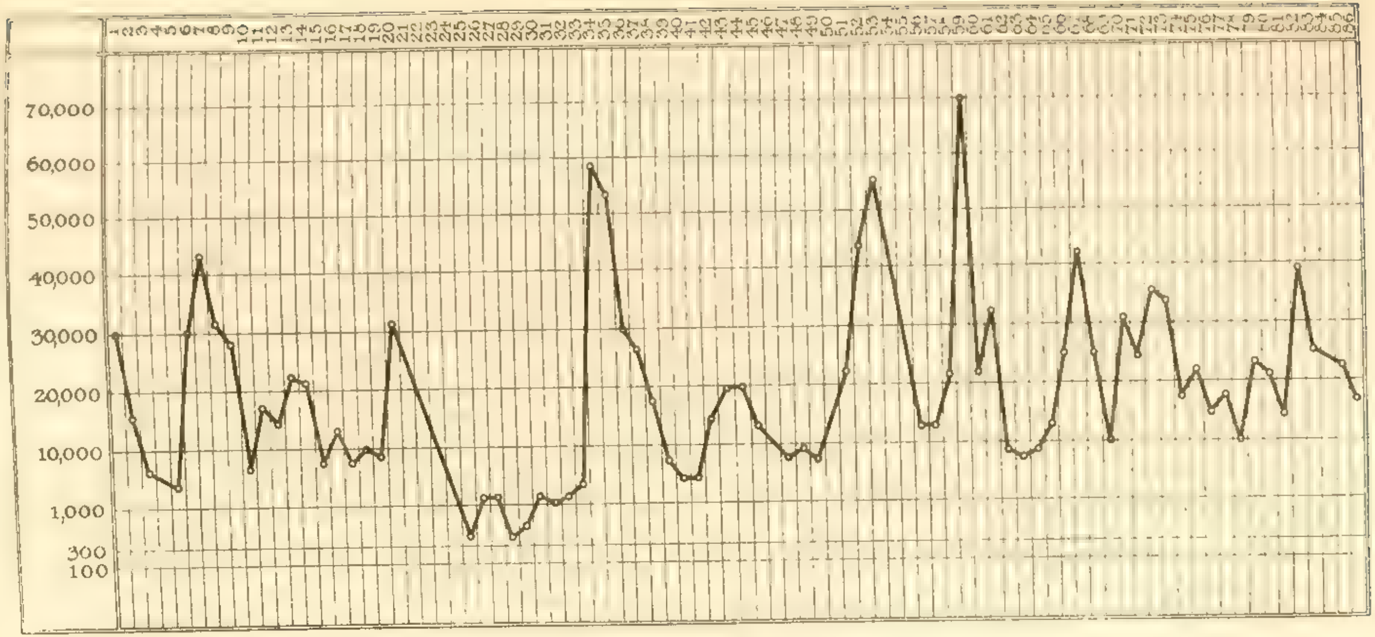

Chart 6.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. gambiense*. Periodic Variation. (See record of Guinea-pig 7.)

[The graph represents the daily numbers of the parasites from the 20th to the 105th day, when death occurred. The days should have been numbered accordingly, and not as 1 to 86.]

Guinea-pig 7—continued.

Day	48	49	50	51	52	53	54
Number of Trypanosomes per c.mm.	552	1,440	1,000	1,232	3,484	58,608	52,38
Temp.		78	76	76	—	72	
Weight in grams . .		—	—	463	—	—	
Leucocytes		—	—	—	—	17,160	[illegible]

Day	55	56	57	58	59	60	61
Number of Trypanosomes per c.mm.	31,124	26,416	18,368	8,112	5,840	5,184	15,650
Temp.	80	78	75	74	74	76	-
Weight in grams	—	—	—	—		—	-
Leucocytes	10,408	12,480	24,832	23,972	15,920	31,644	22,116

Day	62	63	64	65	66	67	68
Number of Trypanosomes per c.mm.	19,244	19,584	13,760	11,360	8,640	9,502	[illegible]
Temp.	73	78	80	80	80	70	[illegible]
Weight in grams				—		—	-
Leucocytes	18,564	18,432		26,960	20,480	41,272	-

Day	69	70	71	72	73	74	75
Number of Trypanosomes per c.mm.	15,800	23,848	42,480	57,240	45,800	30,100	12,144
Temp.	77	80	80	86	84	80	80
Weight in grams	—		-	500	-	—	—
Leucocytes (local)		12,672	25,040	15,480	14,560	—	-

Guinea-pig 7—continued.

Day	76	77	78	79	80	81	82
Number of Trypanosomes per c mm. . ..	12,180	21.328	70,000	21,160	32,944	9,048	7,392
Temp.	80	80	80	86	84	84	84
Weight in grams	—	—	—	—	—		
Leucocytes		—	—	—			

Day	83	84	85	86	87	88	89
Number of Trypanosomes per c mm.	9,056	12.760	25,632	42,000	25.000	10,570	31,680
Temp.	—	86	85	80	—	—	
Weight in grams		—	—	513			—
Leucocytes						—	—

Day	90	91	92	93	94	95	96
Number of Trypanosomes per c.mm.	24,180	35,112	34,368	17,248	21,648	13,832	17.920
Temp.	82		—		—	—	—
Weight in grams			—			—	
Leucocytes	—		—	—	—	—	—

Day	97	98	99	100	101	102	103
Number of Trypanosomes per c.mm.	10,248	23,832	21,888	13,072	39,128	25,576	
Temp.	—	—		—	—	—	
Weight in grams	—	—	—	—	—	—	
Leucocytes	—	—	—	—		—	

Day	104	105	—	—	—	—	
Number of Trypanosomes per c.mm.	23,056	17,848	Died	—	—	—	
Temp.			—	—	—	—	—
Weight in grams	—		—	—	—	—	
Leucocytes		—	—			—	—

The graph of Guinea-pig 7 is represented in Chart 6.

Guinea-pig 8, ♂.—Weight 867 grams at time of inoculation; 830 grams on 92nd day; 755 grams at death. Dose of inoculation: 500,000 Trypanosomes. Duration of life 130 days. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.					8	4	Interval of 38 days' vacation
Temp.		3			35	36	
Day	**45**	**46**	**47**	**48**	**49**	**50**	**5**
Parasites per c.mm.	7,540	8,640	5,808	13,156	14,400	18,000	1,2
Temp.	26	16	20	44	20	55	20
Day	**52**	**53**	**54**	**55**	**56**	**57**	**58**
Parasites per c.mm.	4,896	1,792	576	11,000	9,600	12,800	18,200
Temp.	34	46	30	33	38	50	46
Day	**59**	**60**	**61**	**62**	**63**	**64**	**65**
Parasites per c.mm.	9,216	1,024	2,916	6,000	7,680	10,000	2,000
Temp.	36	54	36	20	34	60	40
Day	**66**	**67**	**68**	**69**	**70**	**71**	**72**
Parasites per c.mm.	1,220	1,440	720	1,624	2,160	5,600	11,520
Temp.	30	46	26	50	52	26	64
Day	**73**	**74**	**75**	**76**	**77**	**78**	**79**
Parasites per c.mm.	6,624	4,480	5,760	3,600	5,760	2.016	2,400
Temp. ...	34	40	50	46	54	30	60

Guinea-pig 8—continued.

Day ...	80	81	82	83	84	85	86
Parasites per c.mm.	6,600	5,600	8,400	12,000	4,032	1,920	2,304
Temp.	20	24	42	36	46	76	72

Day	87	88	89	90	91	92	93
Parasites per c.mm.	2,184	2,160	3,920	7,840	4,608	3,360	5,040
Temp.	68	69	66	64	64	68	70

Day	94	95	96	97	98	99	100
Parasites per c.mm.	3,920	4,200	6,400	5,960	2,268	2,160	5,760
Temp	84	64	70	62	60	64	62

Day	101	102	103	104	105	106	107
Parasites per c.mm.	15,680	19,584	9,792	18,000	36,960	5,400	9,600
Temp.	58	56	54	70	74	72	66

Day	108	109	110	111	112	113	114
Parasites per c.mm.	18,000	17,920	14,400	16,000	19,600	8,640	15,680
Temp.	72	74	66	70	76	72	80

Day ..	115	116	117	118	119	120	121
Parasites per c.mm.	23,040	5,600	9,600	10,240	6,400	12,800	10,240
Temp.	64	74	70	56	76	60	70

Guinea-pig 8—continued.

Day	122	123	124	125	126	127	128
Parasites per c.mm.	16,800	9,600	16,364	25,088	864	7,200	[illegible]
Temp.	70	80	74	88	74	82	74

Day	129	130	—	—	—	—	—
Parasites per c.mm.	900	15,000	Died	—	—	—	
Temp.	86	—	—	—	—	—	—

Guinea-pig 9, ♀.—Weight 529 grams at time of inoculation; 440 grams at death. Dose of inoculation: 100,000 Trypanosomes. Duration of life 96 days. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—			—	4	8	Interval of 38 days vacation
Temp.		30			24	35	

Day	45	46	47	48	49	50	51
Parasites per c.mm.	28	36	84	72	228	240	256
Temp.	32	20	31	24	22		[illegible]

Day	52	53	54	55	56	57	58
Parasites per c.mm.	396	1,820	720	1,350	7,500	7,840	10,920
Temp.	30	20	20	28	38	20	[illegible]

Day	59	60	61	62	63	64	65
Parasites per c.mm.	15,340	10,368	7,680	8,064	15,680	16,800	[illegible]
Temp.	41	26	38	32	32	12	40

Guinea-pig 9—continued.

Day	66	67	68	69	70	71	72
Parasites per c.mm.	3,864	7,920	3,600	1,700	2,880	5,840	8,400
Temp.	30	35	30	25	30		44

Day	73	74	75	76	77	78	79
Parasites per c.mm.	12,800	9,600	7,200	3,000	8,640	9,200	8,400
Temp.	40	30	28	40	34	36	40

Day	80	81	82	83	84	85	86
Parasites per c.mm.	67,648	59,860	36,800	27,000	10,080	5,880	3,072
Temp.	30	20	26	42	20	70	74

Day	87	88	89	90	91	92	93
Parasites per c.mm.	5,120	7,840	3,600	6,272	5,400	6,720	25,600
Temp.	72	70	44	56	62	70	5

Day	94	95	96	
Parasites per c.mm.	14,400	11,200	42,000	Died
Temp.	26	[illegible]	0	

Guinea-pig 10, ♀.—Weight 495 grams at time of inoculation, 402 grams on 100th day, 335 grams at death. Dose of inoculation: two million Trypanosomes. Duration of life 147 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.						—	
Temp. . .	50		45			50	

Guinea-pig 10—continued.

Day	8	9	10	11	12	13	[illegible]
Parasites per c.mm.	4	0	0	52	4	4	16
Temp.	56	26	46	50	40	50	

Day	15	16	17	18	19	20	[illegible]
Parasites per c.mm.	200	16	4	4	4	12	[illegible]
Temp.	34	50	40	31	26	13	[illegible]

Day	22	23	24	25	26	27	[illegible]
Parasites per c.mm.	4	56	8	4	4	24	[illegible]
Temp.	23	44	20	24	28		[illegible]

Day	29	30	31	32	33	34	35
Parasites per c.mm.	4	4	8	4	20	20	8
Temp.	27	18	50	51	20	53	40

Day	36	37	38	39	40	41	42
Parasites per c.mm.	4	4	16	8	76	8	8
Temp.	36	36	31	34		26	34

Day	43	44	45	46	47	48	49
Parasites per c.mm.	0	0	0	0	24	20	480
Temp.	34	30	38	34	32	26	

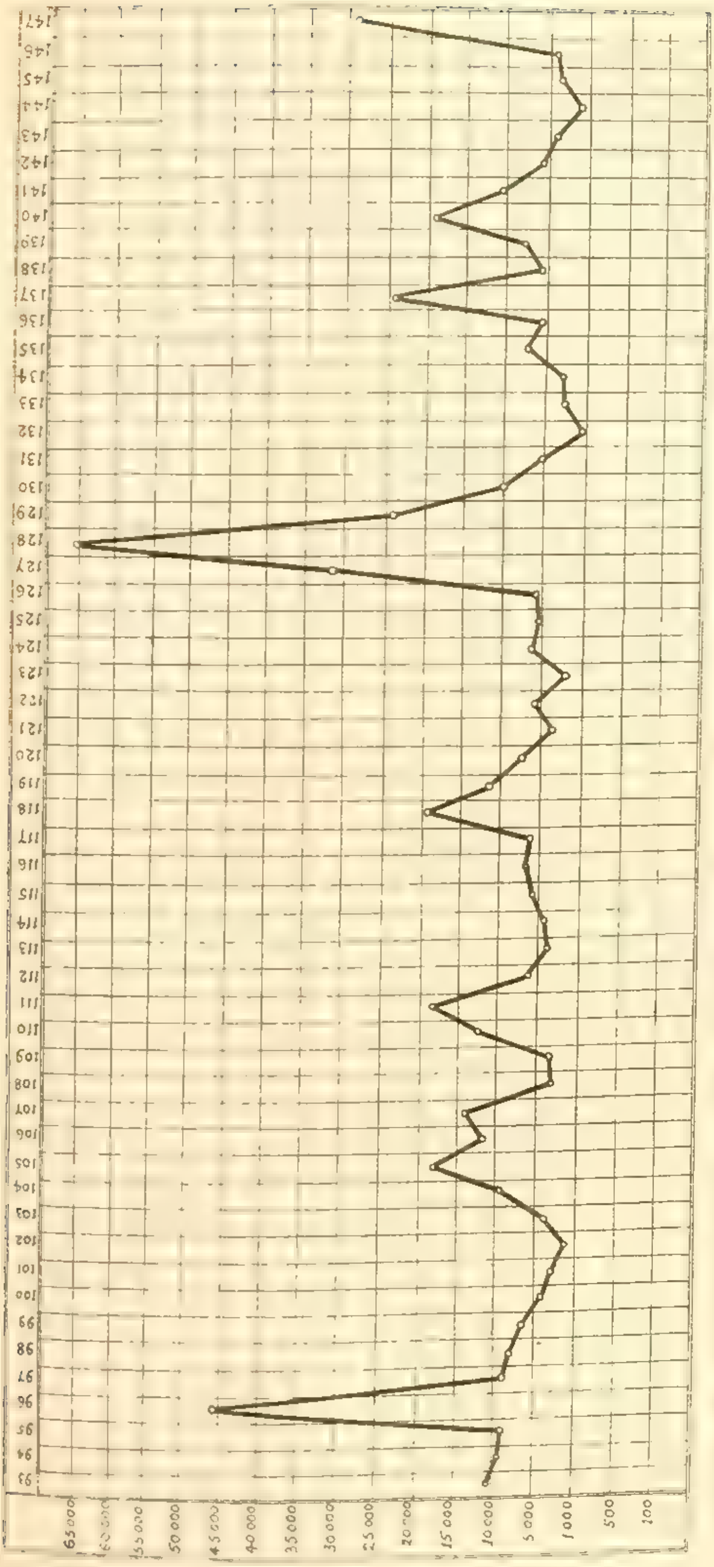

Chart 7. Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. gambiense*. **Periodic Variation.** (See record of Guinea-pig 10.)

Guinea-pig 10—continued.

Day … … …	50	51	52	53	54	
Parasites per c.mm. … …	1,880	416	124	368	116	Interval of 38 days' vacation
Temp. … … …	40	34	23	32	15	

Day … … …	93	94	95	96	97	98	99
Parasites per c.mm. … …	11,240	9,216	8,064	46,180	8,096	7,920	6,19
Temp. … … …	34	44	30	32		34	42

Day … … …	100	101	102	103	104	105	106
Parasites per c.mm. … …	4,864	3,300	2,000	4,576	9,240	18,200	12,4
Temp. … … … …	34	46	30	32		51	31

Day … … …	107	108	109	110	111	112	113
Parasites per c.mm. … …	14,400	3,168	3,840	12,800	18,600	6,160	3,920
Temp. … …	20	50	42		60	50	52

Day … … …	114	115	116	117	118	119	12
Parasites per c.mm. … …	4,536	5,040	6,912	6,000	19,200	12,000	02
Temp. … … … …	50	32	25		48	35	22

Day … … …	121	122	123	124	125	126	127
Parasites per c.mm. … …	3,520	5,600	2,688	6,400	5,040	5,728	32,0
Temp. … … …	40	30	44	38	36	40	40

Guinea-pig 10—continued.

Day	128	129	130	131	132	133	134
Parasites per c.mm.	66,000	24,500	10,000	5,040	1,008	3,600	3,888
Temp.	30	22	40	33	54	44	63

Day	135	136	137	138	139	140	141
Parasites per c.mm.	7,680	5,760	24,000	5,760	7,680	19,440	11,880
Temp.	44	70	44	70	78	72	96

Day	142	143	144	145	146	147	
Parasites per c.mm.	6,480	4,860	2,268	3,888	4,480	28,880	Died
Temp.	80	76	30	52	14	0	

The graph of Guinea-pig 10 is represented in Chart 7.

The incubation period in the case of guinea-pigs varies so much in both strains that it is impossible to draw any definite conclusions therefrom.

The duration of life of guinea-pigs inoculated with the Rhodesian strain was shorter than in those similarly inoculated with *T. gambiense* (old laboratory strain); thus, the average life of the guinea-pigs inoculated with *T. rhodesiense* was 59 days, while the life of guinea-pigs inoculated with *T. gambiense* averaged 111 days.

There is a tendency for the period between the crests of the waves in the graphs of guinea-pigs to be slightly greater in the case of *T. gambiense* than in the case of *T. rhodesiense*.

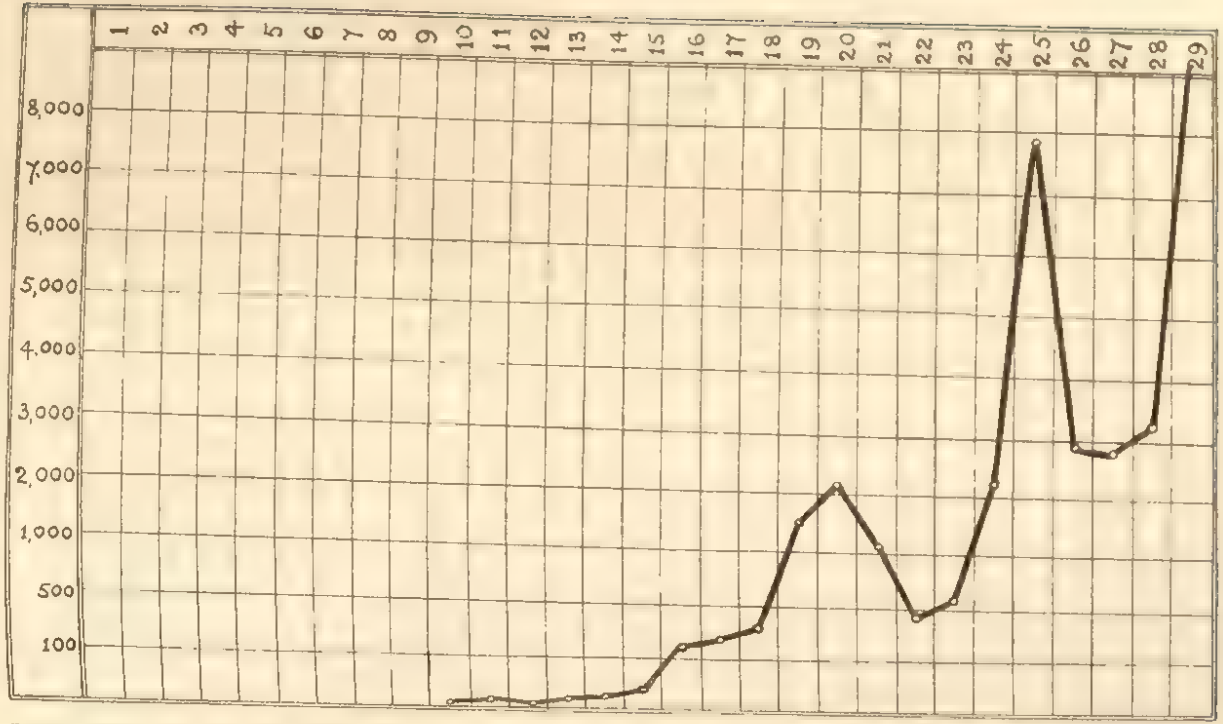

CHART 8.- Graph of Daily Counts of the Parasites in the Peripheral Blood of a Rabbit inoculated with *T. rhodesiense*. Periodic Variation (See record of Rabbit 1.)

ENUMERATIONS IN RABBITS

Two rabbits were inoculated with *T. rhodesiense* and the course of the infection was followed in them, the same methods being employed as in previous cases. We append tables:—

T. rhodesiense

Rabbit I.—Grey and white. Dose of inoculation: 600,000 Trypanosomes, intraperitoneally. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm. ..							
Day	8	9	10	11	12	13	14
Parasites per c.mm.			2	4	1	6	8
Day	15	16	17	18	19	20	21
Parasites per c.mm.	32	168	200	360	1,500	2,184	1,008
Day	22	23	24	25	26	27	28
Parasites per c.mm.	400	672	2,200	7,960	2,944	2,880	3,360
Day	29	–	–		–	–	–
Parasites per c.mm.	35,200	Died	–	–	–	–	–

The graph of Rabbit 1 is shown in Chart 8.

Rabbit 2.—Grey. Dose of inoculation: 600,000 Trypanosomes, intraperitoneally. (J.G.T.)

Day	1	2	3	4	5	6	[illegible]
Parasites per c.mm. .. .	—	—		—	—	—	—

Day	8	9	10	11	12	13	14
Parasites per c.mm. . ..	—		2	8	12	48	[illegible]

Day	15	16	17	18	19	20	21
Parasites per c.mm.	15	1	4	20	100	160	420

Day	22	23	24	—	—	—	—
Parasites per c.mm.	1,776	2,320	2,184	Died	—	—	—

COMPARISON OF THE EFFECTS OF THE TRYPANOSOMES ON THE VARIOUS HOSTS

In guinea-pigs the disease tended to run a more chronic course than in rats, as shown by the fact that the animals lived much longer. The periods between the crests of the waves in both strains in guinea-pigs were longer than in rats, namely five to eight days in most cases. Further, in guinea-pigs the initial rise is slower—a 'staircase' effect taking place and leading to the chronic condition (see Charts 4, 5).

Some of the guinea-pigs when the disease became chronic showed an alternation of high and low rises, which simulate remarkably the graph of the parasites of the patient W.A. (see R. Ross and D. Thomson (1910)*).

In the two rabbits used it was remarkable that these were the only animals that exhibited an affection of the eyes and skin. A white purulent discharge occurred from the eyes in both rabbits about the seventh day after inoculation. Simultaneously with this affection of the eyes the skin at the roots of the ears became encrusted with scabs. Both rabbits became drowsy early in the disease, and remained so until death. Although the incubation period in the case of rabbits was long (nine days), yet the animals early succumbed to the effects of the disease, in spite of the fact that the trypanosomes were never very numerous in the peripheral blood.

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* Proc. Roy. Soc., B, Vol. LXXXII, p. 413.

Rats also showed drowsiness during the latter half of the infection, while guinea-pigs continued to be lively and to take food well until a day or so before death, when in some cases drowsiness ensued.

In our experiments we did not observe any marked relation between the colour of the animal inoculated and the duration of infection in that animal. (It is sometimes thought that white rats are less resistant than piebald.)

In passing it may be noted, as has been done before, that the temperature of the host tended to rise with increase in the number of trypanosomes in the peripheral blood.

EXPLANATION OF THE PERIODIC INCREASE AND DECREASE IN THE NUMBERS OF TRYPANOSOMES IN THE PERIPHERAL BLOOD OF THE HOST

We believe that periodic increase and decrease of the numbers of trypanosomes in the peripheral blood of the host is a natural phenomenon, resulting from the reaction of the host and parasite on each other.

The parasites increase by longitudinal division until a maximum is reached when, unfavourable conditions arising, the formation of rounded, latent, non-flagellate bodies occurs, especially in the internal organs of the host where they collect in the spleen and bone marrow during the period of decrease of flagellates in the peripheral blood. These rounded bodies, or at least some of them, become flagellate trypanosomes when the parasites begin to increase in numbers again (see Fantham (1911)†).

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† Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227, and Ann. Trop. Med. and Parasitol., Vol. IV, pp. 465-485.

The slight fluctuations in the periodicity, namely, the variations in the time-intervals between the crests of the waves, can be explained by the fact that the resistance of the animal varies from time to time. Again, we might note that the rat, the guinea-pig and the rabbit are not the natural hosts of the parasites of sleeping sickness.

We should also note that the strains with which we have been dealing are very virulent, as judged by the records of Thomas and Linton (1904),* Moore and Breinl (1907), and others, whose inoculated animals lived much longer than ours. In less virulent strains the time-intervals between the crests of the waves in the graphs might tend to be longer.

The periodic variation or oscillation that we have described is of practical importance in the administration of drugs to eliminate trypanosomes in a host. If the drug be administered on the downward slope of the curve, the fall in numbers of trypanosomes is not due to the drug alone. But if the drug be administered on the upward slope of the curve, while the parasites are increasing in numbers, and yet a reduction in numbers of the parasites is brought about, then the drug may legitimately be inferred to be efficacious.

SUMMARY

1. In these investigations two strains of trypanosomes were used, namely, (*a*) *T. rhodesiense* (Stephens and Fantham) derived from a patient, W.A., suffering from sleeping sickness contracted in Rhodesia, and (*β*) *T. gambiense*, an old laboratory strain.

2. Of these two strains the Rhodesian was the more virulent, as judged by sub-inoculations in rats, guinea-pigs and rabbits, for the duration of life of animals inoculated with the Rhodesian strain was shorter than in those inoculated with *T. gambiense*.

Stephens and Fantham (1910) have already pointed out a distinct morphological peculiarity in *T. rhodesiense* in the presence of a posterior nucleus in some of the stout forms.

3. Enumeration of trypanosomes in the peripheral blood of rats, guinea-pigs and rabbits inoculated with each of these strains

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* Lancet, May 14, 1904, pp. 1337-1340.

reveals remarkable variations or oscillations in the numbers of the parasites from day to day, the variations being of the nature of a periodic increase and decrease at more or less regular intervals.

4. The periodicity is to be explained by (*a*) variations in resistance on the part of the host, probably due to the formation of anti-bodies, accompanied by (*β*) the formation on the part of the trypanosomes of rounded, latent, non-flagellate (relatively resistant) forms in the internal organs of the host during the fall in numbers of the flagellate parasites in the peripheral blood.

There is a life-cycle of trypanosomes in the Vertebrate host, in addition to stages of the parasite in the Invertebrate carrier (for example, *Glossina*).

THE LIFE-HISTORY OF *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* AS SEEN IN RATS AND GUINEA-PIGS*

BY

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(*Received for publication* 15 *October*, 1910)

Plate XXVII.

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INTRODUCTION

The researches recorded in this paper were undertaken at the suggestion of Major Ross, who wished me to investigate the parasitological aspect of the numerical cyclical development discovered by him and Dr. D. Thomson (1910) in the trypanosome occurring in a patient suffering from sleeping sickness contracted in Rhodesia, particularly as regards the possible connection of the latent bodies of Salvin-Moore and Breinl (1907) with that cycle. The investigations have been conducted in the Liverpool School of Tropical Medicine, under a grant from the Tropical Diseases Research Fund.

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* Read before the Royal Society on December 8, 1910, and reprinted from Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227.

A complete and generally accepted life cycle of *Trypanosoma gambiense* has yet to be written. The following paper is offered as a contribution to the solution of this difficult problem, and deals with that portion of the life history of the parasite which takes place in a vertebrate host.

The sub-inoculations in animals—rats and guinea-pigs—recorded herein were made from a patient suffering from Rhodesian sleeping sickness in Professor Ross's clinic in the Royal Southern Hospital Liverpool. The trypanosome from this source showed a marked morphological feature in the possession of a posterior nucleus in some forms, and for this parasite the name *T. rhodesiense* has been suggested by Stephens and Fantham (1910). A laboratory strain of *T. gambiense* was also used in these investigations for comparison.

Special attention has been paid to the observation of the *living* parasite, as well as to stained preparations.

METHODS

Fresh preparations of *T. gambiense* and *T. rhodesiense* were made from blood taken aseptically from animals, namely, tame rats and guinea-pigs. The blood was kept in sealed cover-slip preparations, at or below blood heat (25° to 37° C.), and also at laboratory temperature. The blood was sometimes diluted with a little physiological salt solution or with a little isotonic sodium citrate solution. Methylene blue was sometimes used for *intra vitam* staining.

Wet preparations of the parasite were made on cover-slips after fixation with osmic acid vapour or corrosive acetic alcohol. The chief stains used were iron haematoxylin and those of Giemsa and Romanowsky.

Dry smears were employed for rough work.

In animals killed at certain stages of infection, the various internal organs were carefully examined both in fresh preparations and stained smears.

The enumerative methods used were those recently employed by R. Ross and D. Thomson and used by various workers in Liverpool, being an elaboration of R. Ross's thick-film method made with measured quantities of blood (1 cubic millimetre divided into quarters. The films were dehaemoglobinised, fixed in absolute alcohol, and stained by the Romanowsky method.

RÉSUMÉ OF PREVIOUS WORK ON LATENT BODIES OF TRYPANOSOMES

The various flagellate forms of *Trypanosoma gambiense* have been so often described by many competent workers that it is needless to discuss them further in detail. Suffice it to say that long, thin trypanosomes may occur, especially at the beginning of infection in rats and guinea-pigs, and shorter, stout or stumpy forms later. Trypanosomes intermediate in character also are found.

The so-called rounded, latent or encysted forms, which are non-flagellate, must be discussed in greater detail. Marked attention to these non-flagellate forms of trypanosome was first drawn by Moore and Breinl (1907-8) in mammalian trypanosomes, though Dutton saw rounded forms of an amphibian trypanosome on the Congo in 1903-5. Moore and Breinl (1907), working on stained material, stated that latent forms occurred in the internal organs, especially during the periods when the flagellates were decreasing or absent from the peripheral blood of the host. These authors give a curve showing the variations in the numbers of *T. gambiense* in infected rats; they do not, however, give any numerical data in support of the graph. A few other workers have mentioned rounded bodies in connection with trypanosomes, among whom Hindle (1909) may be noted. The significance of the latent bodies is still a disputed point, and it was recently stated in a review that more evidence was required to show that they 'constitute part of a life-cycle in the vertebrate host.'*

In the present paper the general statements of Moore and Breinl, regarding forms of *T. gambiense* in the internal organs of rats, are shown to be accurate, and many details, as seen in the living parasite, as well as numerical data from daily counts of the trypanosomes, are supplied in proof of the significance of the latent bodies. Animals inoculated with *T. rhodesiense* (Stephens and Fantham) have also been carefully investigated.

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* S.S. Bureau, Bulletin No. 15 (March, 1910), p. 102.

THE RELATION BETWEEN LATENT BODIES AND THE NUMBER OF TRYPANOSOMES IN THE PERIPHERAL BLOOD, WITH NOTES ON THE PARASITES FOUND IN THE INTERNAL ORGANS OF THE HOSTS

Following on the work of R. Ross and D. Thomson (1910) on the enumeration of the parasites in the peripheral blood of a patient suffering from Rhodesian sleeping sickness, the periodic increase in the number of trypanosomes in the peripheral blood of rats and guinea-pigs inoculated with *T. rhodesiense* or with *T. gambiense* has recently been found by Fantham and J. G. Thomson.

Rats similarly inoculated and exhibiting such periodic increase and decrease were killed at various points in the cycle, as set forth in the following tables:—

A. Rat 1.—*T. gambiense*, Laboratory Strain, in Piebald ♂ Rat, weight 150 grams, inoculated with 50,000 trypanosomes.

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—		4	[illegible]
Temp.*		55	46	70		62	57	54

Day	9	10	11	12	13	14	15
Parasites per c.mm.	4392	868	1656	2560	9288	6304	436, killed
Temp.*	60	68	44	46	57	54	61

* The temperature is expressed according to the following convention, as in Major Ross's recent papers on 'Malaria' and 'Trypanosomiasis': -Temp. = (F − 95) × 10, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer.

Heart blood of Rat 1 examined fresh, immediately after killing.—A few living trypanosomes seen and some (fewer) rounded forms.

Spleen.—Very large; three to seven rounded forms seen in every field of the microscope (2 mm. objective and 2 ocular); no flagellates seen in fresh preparation.

Liver and portal blood.—Living flagellates and rounded bodies seen in about equal numbers (one of each per field).

Lungs.—A few living flagellates seen, four times as many rounding and rounded bodies seen [100 parasites counted in this and subsequent fresh preparations, when possible].

Bone marrow.—Many rounded bodies.

RAT 2.—*T. gambiense*, Laboratory Strain, in White ♀ Rat, weight 128 grams, inoculated with 200,000 trypanosomes.

Day	1	2	3	4	5	6	7
Parasites per c.mm.					4	684	3640
Temp.	38		40	45	50	54	60

Day	8	9	10	11	12
Parasites per c.mm.	7392	[illegible]	6320	1840	2200, killed
Temp.	55	58	44	46	48

The internal organs of Rat 2 were in much the same condition as in Rat 1; further details are superfluous.

B. RAT 3.—*T. rhodesiense* in Piebald Rat, ♀, weight 108 grams, inoculated with 350,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	172	18,160	42,120	31,040
Temp.	22	20	20	40	56	36

Day	7	8	9	10
Parasites per c.mm.	21,600	12,844	130,000	61,440, killed
Temp.	20	21	15	14

Heart blood, fresh, of Rat 3.—Free trypanosomes seen.

Spleen.—Many rounded bodies; no free trypanosomes seen.

Liver.—A few free trypanosomes seen.

Lungs.—About equal numbers of flagellates and rounded bodies.

Bone marrow.—Rounded bodies.

RAT 4.—*T. rhodesiense* in White Rat, ♂, weight 120 grams, inoculated with 600,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—		4	866	288[illegible]	3136
Temp.		20	25	38	50	50

Day	7	8	9	10	1
Parasites per c.mm.	51,2[illegible]	64,000	22,820	12,500	6144, killed
Temp.	48	47	13	2[illegible]	31

Heart and liver of Rat 4.—Many large trypanosomes seen in fresh preparations, very few rounded bodies.

Lungs.—A few free flagellates, three times as many rounded forms.

Spleen.—A few small flagellate trypanosomes (3 per cent.; many rounded forms.

Kidney blood.—Flagellate trypanosomes.

Bone marrow.—Rounded bodies.

RAT 5.—*T. rhodesiense* in White Rat, ♀, weight 247 grams, inoculated with 120,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.		.	—	—	4	12
Temp.			52	38	34	96

Day	7	8	9	10
Parasites per c.mm.	20	296	624	3744, killed
Temp.	86	86	20	66

Flagellate trypanosomes were seen in all the internal organs of Rat 5. A few latent bodies were seen in the lungs, spleen, and bone marrow.

Rat 6.—*T. rhodesiense* in White Rat, ♂, weight 150 grams, inoculated with 500,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9
Parasites per c.mm.	—	—	—	—	72	2056	1968	6420	592, killed
Temp.	38	36	51	56	54	70	70	62	54

Heart blood, fresh, of Rat 6.—Flagellate parasites seen, only three latent bodies found in a fresh preparation.

Liver.—Flagellates 96, and latent bodies 4; in one hour, however, the number of latent bodies in the fresh preparation had increased 16 times (namely to 64), and the flagellates were correspondingly fewer.

Lungs.—About equal numbers of trypanosomes and latent bodies.

Spleen.—Many latent bodies, no free flagellates seen.

Bone marrow.—A few latent bodies, no flagellates seen.

Rat 7.—*T. rhodesiense* in Piebald Rat, ♀, weight 170 grams, inoculated with 1,000,000 trypanosomes. Weight 182 grams at death, but pregnant.

Day	1	2	3	4	5	6
Parasites per c.mm.			8	5852	27,916	16,468, killed
Temp.	38		62	55	67	66

Heart blood of Rat 7.—Many flagellates, few rounded bodies.

Liver.—Equal numbers of flagellates and rounded bodies.

Lungs.—Three times as many latent bodies as flagellates.

Bone marrow.—Large rounded bodies.

Spleen.—No flagellates seen, but many rounded bodies.

Splenic vein blood.—Twice as many flagellates as rounded bodies.

Mesenteric gland fluid.—Many flagellates.

Lymph.—Rounded bodies and Crithidia-like forms.

Placental blood.—Living flagellates, few rounded bodies.

Thirteen embryos.—Serous fluid contained a very few trypanosomes.

Embryonic liver.—No flagellate trypanosomes seen.

From counts of the number of trypanosomes in the peripheral blood daily, and from examination of carefully prepared smears of organs, it is found that latent bodies are most numerous when the flagellate parasites are few. If inoculated animals be killed at these periods, very few flagellate trypanosomes are found in the spleen and bone marrow (see preceding tables), but many latent bodies are present in those organs, while rounding forms are seen especially in the lungs.

In the peripheral blood, on the upward slope of the curve representing the numbers of the parasites from day to day, the parasites increase in numbers by longitudinal division to a maximum. At or about this period the formation of rounded or latent bodies begins, and takes place especially in the internal organs.

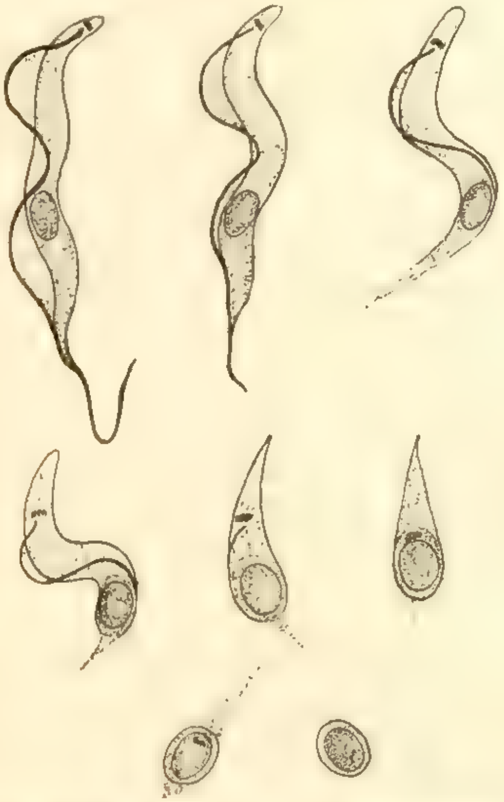
If rounded (latent) bodies, derived from the internal organs of an infected rat, be placed in warm fresh blood drawn from a normal, uninfected rat, then growth of some of the rounded bodies towards the flagellate trypaniform stage can be seen under the microscope, as is detailed in a subsequent section of this paper.

Five guinea-pigs (three inoculated with *T. rhodesiense* and two with *T. gambiense*), dying in various stages of trypanosomiasis were carefully examined, and fresh preparations and smears of their internal organs were made. Rounding and rounded forms of trypanosomes were seen, just as in infected rats.

Needless to say, a very careful examination of the internal organs of normal (uninfected) rats and guinea-pigs was made for the purposes of comparison and control.

THE FORMATION OF LATENT BODIES FROM FLAGELLATE TRYPANOSOMES

This is diagrammatically represented in text-fig. 1, in which the formation of a rounded body from a living trypanosome was observed under the microscope in a drop of blood and lymph from the tail of an infected rat. The time taken for the formation of the rounded body was thirty minutes.



Text-fig. 1 represents diagrammatically the formation of a latent (non-flagellate) body of *Trypanosoma gambiense* in infected rat's blood as seen under the microscope, on a warm stage, during a period of 30 minutes.

The intervals were 3, 8, 15, 20, 23, 25, and 30 minutes respectively from the commencement of change of form of the parasite. Intranuclear karyosome not represented.

In this process the anterior or flagellar end disintegrates and is cast off, while at the other end the blepharoplast (kinetonucleus) gradually migrates nearer the nucleus, and then the non-flagellar or posterior end of the original trypanosome is cast off, together with the remains of the flagellum. The rounded body, consisting of chromatin with a thin layer of cytoplasm, has then surrounded itself with a definite, very thin capsule ('cyst'). This process, as seen under the microscope, is either the natural mode of formation of non-flagellate bodies in the internal organs or closely approximates thereto, as intermediate stages exactly similar in the formation of these bodies are seen in stained preparations of the heart, lungs and spleen (Plate XXVII, figs. 1-5).

However, in the peripheral blood there occur rounded, oval or somewhat pyriform parasites (figs. 17-20), each with a single anterior flagellum. Such forms may, for convenience, be called rounding herpetomonad forms, as *Herpetomonas* passes through similar stages in assuming a rounded non-flagellate form. The rounded stages of *Herpetomonas*, thus formed, have been aptly termed post-flagellate stages by Captain Patton and by Dr. Annie Porter in their recent interesting researches on flagellates (*Crithidia* and *Herpetomonas*). The rounded, encapsuled stages of trypanosomes are post-flagellate stages. That these rounded post-flagellate forms are encapsuled in a thin membranous structure is shown by the fact that they resist maceration in water much longer than the trypaniform flagellates.

The formation of post-flagellate bodies (figs. 2, 3, 5) is well seen in the lungs, whence they find their way in the blood stream to the spleen and bone marrow.

THE STRUCTURE OF LATENT BODIES

The post-flagellate (latent) stages of *T. gambiense* and *T. rhodesiense*, already mentioned, have a relatively simple structure. The strictly latent or non-flagellate forms are usually oval in outline, and small, about 2 μ to 4 μ in diameter (figs. 2-16). Less frequently are they quite rounded or spherical (fig. 14), while sometimes they are pyriform (figs. 25, 27, 29, 30). Internally there is a nucleus, which may show a karyosome, and beside the nucleus there is a blepharoplast or kinetonucleus (fig. 6). In some latent bodies, especially the smallest ones, the kinetonucleus may not be visible separately (figs. 11, 13-15), as it may be lying over the nucleus, or actually affixed thereto (figs. 2, 10). The juxtaposition of the nuclear bodies has been actually observed in some Romanowsky-stained specimens, after careful wet fixation (figs. 2, 10). The relative positions of the nucleus and blepharoplast in rounding or rounded bodies may vary considerably. A small quantity of cytoplasm occurs in the latent bodies.

In the formation of rounded, latent bodies, as seen *in vitro*, a portion of the body of the flagellate, after passage of the blepharoplast towards the nucleus, is thrown off, and the flagellum is discarded (text-fig. 1). Examination of preparations of the internal organs (lungs, heart, spleen) of the host shows that a similar method of formation of the post-flagellate stages of the parasite usually occurs in the internal organs (figs. 1-5). However, in the peripheral blood, after careful searching of a sufficient quantity of blood (¼ cubic millimetre), a few rounding or rounded parasites can generally be seen (figs. 12, 17-20). Also, in the internal organs, stages of the parasite intermediate between the flagellate and non-flagellate forms may be seen (fig. 33).

The post-flagellate or latent bodies vary in size (figs. 2-16, 22). This variation is due to two causes: (1) the formation of non-flagellate parasites from trypanosomes of different breadths, and (2) the occasional division of large post-flagellate forms by binary fission, an example of the simplest schizogony (fig. 23). This fission, so far as my researches go, is infrequent in the case of *T. gambiense* and *T. rhodesiense*. It has been observed that division of flagellate trypanosomes may immediately precede the formation of latent bodies.

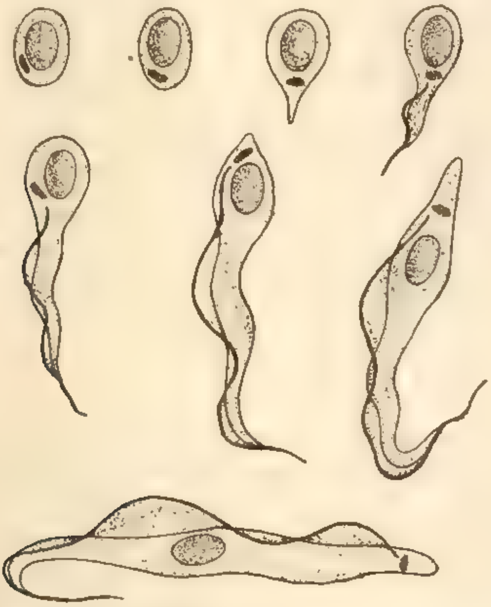
Broad forms of *T. rhodesiense*, with posterior nucleus, may form relatively large latent bodies. As the nucleus is at or near the posterior (non-flagellar) end of the parasite, there is little of the body discarded in that region when rounding occurs. The kidney shape of the nucleus of some specimens of *T. rhodesiense* is seen in their latent bodies (fig. 27), and the nucleus lies to one side of the rounding body (figs. 17, 18). It is not easy, however, to differentiate between the latent bodies of *T. rhodesiense* and *T. gambiense*.

Moore and Breinl describe a stainable band or black line connecting the nucleus and blepharoplast of certain specimens of *T. gambiense* at or near the maxima. An 'interaction' takes place between the blepharoplast and nucleus. After this the formation of latent bodies proceeds. During the researches now recorded, there was no good evidence found in support of Moore and Breinl's views. The stainable band was seen in some stout (probably old) parasites, and in parasites at periods near the death of the host.

It is possible, as Swellengrebel (1908) suggested, that the stainable line is a form of degeneration. However, on the exact significan of the stainable band seen in such trypanosomes I prefer not t pronounce a definite opinion at present.

THE METAMORPHOSIS OF LATENT BODIES INTO TRYPANOSOMES

This process was observed in life on several occasions, thoug the complete passage from a rounded body to a fully flagellate moving trypanosome was only rarely seen (three times), for it i difficult to imitate precisely the natural conditions favourable t such a metamorphosis. However, by taking rounded bodies, usual obtained from the spleen of an infected rat, in a little physiological salt solution, and adding thereto an equal quantity of fres (normal, uninfected) rat's blood, some of the rounded bodies were seen on a warm stage (25° to 35° C.) to grow, each becoming large and sending out a process (text-fig. 2). This pseudopodium-like process lengthens, and a flagellum is formed from an area close t



Text-fig. 2 represents diagrammatically the metamorphosis of a rounded, latent or non-flagella parasite into a flagellate trypanosome (*T. gambiense*). The rounded bodies, obtained fro the spleen of an infected rat, were placed in warm, fresh, uninfected rat's blood and watche under the microscope. The total time taken for the metamorphosis was about one hour.

the blepharoplast (kinetonucleus). At this stage the parasite forms at its drawn-out anterior end an undulating membrane along the edge of which the flagellum lies, and the organism somewhat resembles a *Crithidia*. This transitory stage may be termed the crithidial stage. The organism grows and the blepharoplast passes posterior to the nucleus, and the trypaniform stage is assumed.

The evidence of stained preparations (figs. 32 to 40) fully supports this mode of formation of flagellate trypanosomes from non-flagellate latent bodies. The latent bodies, which are the post-flagellate stages of one generation of trypanosomes, become the pre-flagellate stages of the succeeding generation of trypanosomes. Such pre-flagellate, Crithidia-like parasites in various stages of metamorphosis may be seen in the peripheral blood of the host (figs. 32, 34 to 42), when the parasites are increasing in numbers therein.

A basal granule (blepharoplast of Minchin) is seen at the base of the flagellum of some stained flagellating parasites (fig. 37).

It may be added that in dealing with an isolated case of an intermediate stage of a parasite between the flagellate and the rounded body, it is sometimes difficult to determine whether the given stage is pre-flagellate or post-flagellate, that is, whether the given parasite is proceeding in development towards the flagellate stage or away from it towards the rounded body.

THE SIGNIFICANCE OF THE NON-FLAGELLATE OR LATENT FORMS OF TRYPANOSOMES. INOCULATION WITH LATENT BODIES PRODUCES TRYPANOSOMIASIS

R. Ross and D. Thomson (1910) report periodic variations in the numbers of the trypanosomes found in the blood of a patient, W. A., suffering from Rhodesian Sleeping Sickness. Fantham and J. G. Thomson (1910) report similar periodic variation in the number of the parasites in the peripheral blood of sub-inoculated animals (rats, guinea-pigs, and rabbits). During the periods of decrease of the parasites in the peripheral blood, I find that latent (non-flagellate) bodies are present in relatively large numbers in the internal organs of the host. The latent bodies are formed at or near the period of maximum increase of the trypanosomes in the peripheral blood. The latent bodies are especially numerous in the spleen and bone marrow on the downward slope of the curve representing the numbers of the parasites in the peripheral blood of the host. Change of the latent forms into trypanosomes takes place on the rise or upward slope of the curve.

There is, of course, a mutual action and reaction of the host and the parasite, the resistance of the host probably being greatest when the flagellate trypanosomes within it are beginning to decrease, thus helping to bring about the assumption of the rounded form by many of the flagellates, so that latent or resistant non-flagellate stages of the parasite are then numerous.

The occurrence of latent bodies also helps to explain the successful inoculation of animals with trypanosomiasis when no flagellates can be found in the blood inoculated from a previously infected animal. Although it might be urged that flagellate trypanosomes in numbers too few to recognise may actually be present in the infected blood inoculated, yet it is possible to inoculate only latent, non-flagellate bodies, and give the inoculated animal trypanosomiasis. In other words, persistent infectivity in the case of trypanosomes is explained by rounded bodies.

I have performed this experiment (inoculation of latent bodies on two occasions. In the first experiment a rat was inoculated with one drop of spleen-pulp (from Rat 7) mixed with a little sterile physiological salt solution, the mixture containing no flagellates The inoculated rat developed trypanosomiasis on the 6th day, dying on the 12th day. The daily counts of this rat were as follows: –

Day	1	2	3	4	5	6	7
Parasites per c.mm.					-	4	864

Day	8	9	10	11	12
Parasites per c.mm.	2196	4308	30,600	10,000	75,2

Secondly, a further experiment was tried, since it was considered that the parasites in one drop of spleen-pulp solution might be too numerous to count accurately, and that a few flagellate trypanosomes might be contained therein, for although a most minute search was made for flagellates in the fluid before inoculation, yet a very few flagellates might have remained undetected. Accordingly, in the second experiment, 1 cubic millimetre of spleen-pulp solution in sodium citrate was inoculated intraperitoneally. Careful examination of samples of the solution used, both fresh and stained, showed no flagellate trypanosomes, but many rounded bodies. The actual fluid used for inoculation also showed no flagellate trypanosomes when microscopically examined fresh. The rat thus inoculated developed trypanosomiasis, parasites being found on the 8th day, and it died on the 18th day. The daily counts of this rat are appended :—

D[illegible]	1	2	3	4	5	6	7	8	9	10	11
Parasites per c.mm.	—	—			—	—		4	960	[illegible]	[illegible]

D[illegible]	12	13	14	15	16	17	18
Parasites per c.mm. ...	49,200	18,200	64	2120	[illegible]	[illegible]	[illegible]

The two rats inoculated with non-flagellate, latent bodies of *T. rhodesiense* showed incubation periods rather longer (5 to 7 days) than usual (2 to 4 days) with rats inoculated with flagellate *T. rhodesiense*.

The occurrence of non-flagellate bodies in the life-cycle of the parasites of sleeping sickness also explains recurrence of trypanosomiasis after it has apparently died out in an infected animal. In such cases the latent bodies are present in the host all the while in such organs as the spleen and bone marrow.

During these researches it has been found that flagellate trypanosomes (500,000 to 2,000,000 in number) inoculated into a rat or a guinea-pig can be detected in the peripheral blood-stream (in 1 cubic millimetre of blood) of the host for some ten to twelve hours, or even eighteen hours after inoculation. During and after this period, that is, during the incubation period of the parasite, a few rounded forms of trypanosomes can be found in a cubic millimetre of peripheral blood.

Biot (1910) writes of the 'revivifying action' of physiological salt solution on trypanosomes (*T. lewisi*), especially in fluid from the liver of a rat dead six and a half days (kept in the cold, unopened). Biot does not explain the phenomenon. However, it is capable of explanation, for in the liver latent bodies of trypanosomes are present, which, under the relatively favourable environment of isotonic salt solution, flagellate and become typical trypanosomes.

In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded, non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages or disintegrate those latent bodies already formed. In this connection the work of B. Moore, Nierenstein, and Todd* on the combined use of salts of mercury and arsenic should be considered.

NOTE ON THE DEGENERATION OF TRYPANOSOMES

All flagellate trypanosomes do not become rounded and form latent, non-flagellate bodies, but some of them degenerate and die. The degeneration may take various forms—some become (α) somewhat irregular and almost amoeboid with pale-staining cytoplasm and vacuoles (figs. 44, 45), others (β) exhibit chromatolysis, wherein the nucleus becomes poor in chromatin and chromatoid granules occur in the cytoplasm (figs. 46-49), while others (γ) exhibit marked vacuolation (fig. 50). Such degenerating forms may be seen in various internal organs of the host, such as the lungs and spleen, especially during the period of formation of latent bodies.

It is also very probable that some of the latent bodies themselves die and do not flagellate, for some shrunken latent bodies, with undifferentiated contents, can be seen in the spleen.

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* Ann. Trop. Med. and Parasitol. (1907), Vol. I. pp. 275-284.

SUMMARY AND CONCLUSIONS

1. Non-flagellate stages of trypanosomes, such as *T. gambiense* (Dutton) and *T. rhodesiense* (Stephens and Fantham), occur.

2. These non-flagellate stages ('latent bodies' of Moore and Breinl) are especially found in the lungs, spleen and bone marrow, during periods of decrease of trypanosomes in the peripheral blood of the host.

3. They are in process of formation at or near the time when the trypanosomes are most numerous in the peripheral blood. The formation of latent bodies takes place especially in the lungs, and they collect in the spleen and bone marrow of the host.

4. In the formation of non-flagellate stages, some of the cytoplasm and the flagellum of the trypanosome are disintegrated. The non-flagellate body contains the nucleus and blepharoplast (kinetonucleus) of the trypanosome.

5. Non-flagellate (latent) bodies can be seen to grow and flagellate, turning into trypanosomes, when placed in fresh, warm, uninfected blood.

6. Latent bodies of *T. rhodesiense*, inoculated into a rat, flagellate and produce trypanosomiasis.

7. The non-flagellate (latent) bodies of trypanosomes (*T. gambiense* and *T. rhodesiense*) are the post-flagellate stages of one generation of trypanosomes and the pre-flagellate stages of the succeeding generation of trypanosomes.

8. There is a life-cycle of trypanosomes (*T. gambiense* and *T. rhodesiense*) in Vertebrate hosts, comparable with those of *Crithidia* and *Herpetomonas* in the alimentary tracts of various Invertebrates. The latent (relatively resistant) stages of trypanosomes occurring in Vertebrates are separate from, and in addition to, stages of the parasite which may occur in the Invertebrate carrier (for example, *Glossina*).

9. In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages, or disintegrate those latent bodies already formed.

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EXPLANATION OF PLATE XXVII.

All figures were outlined with Abbé-Zeiss camera lucida, using 2 mm. apochromatic homogeneous immersion objective and compensating oculars 8 and 12 of Zeiss. Magnification 2,000 diameters approximately, except where otherwise stated.

The chief stains used were iron haematoxylin, Giemsa, or Romanowsky after wet fixation.

The figures represent *T. rhodesiense*, as seen in rats, except where otherwise indicated.

Fig. 1 represents the formation of latent (non-flagellate) body from trypanosome in heart blood.

Figs. 2-5 represents latent bodies, with remains of trypanosomes around or near, from heart (fig. 4) and lungs.

Figs. 6, 7.—Oval latent bodies, each with nucleus and blepharoplast (kinetonucleus), from lung of rat and peripheral blood of guinea-pig respectively.

Figs. 8-16.—Various forms of latent bodies—from liver, heart, spleen, lung (figs. 8-11); of *T. gambiense* in peripheral blood of rat (fig. 12); from peripheral blood (fig. 13), lung, and heart respectively.

Figs. 17-20.—Rounding, herpetomonad-like forms, all from peripheral blood of guinea-pigs. Fig. 19 of *T. gambiense.*

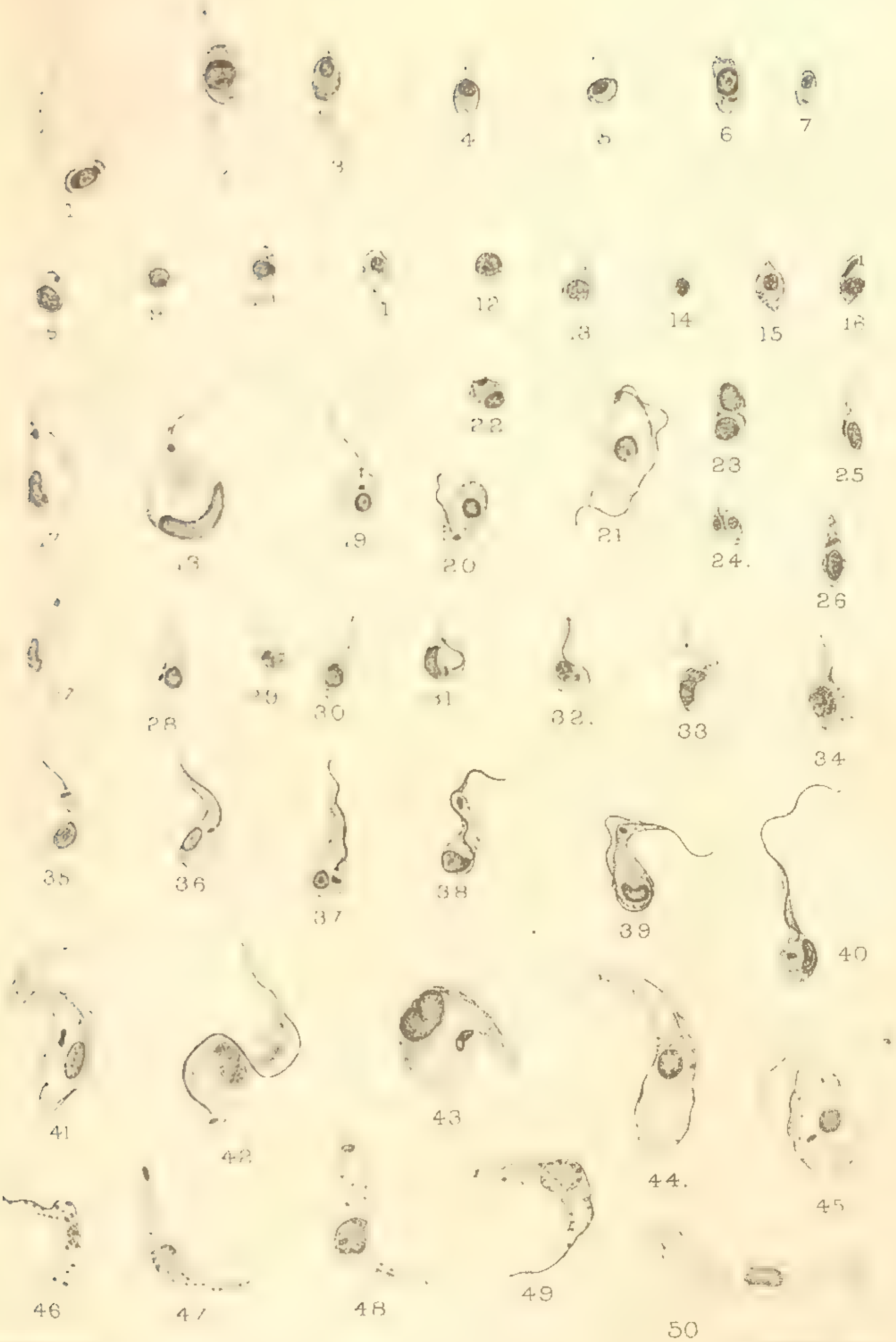
Fig. 21.—Broad trypaniform parasite from blood of guinea-pig.

Fig. 22.—Latent form of *T. gambiense* from lung of rat.

Figs. 23-24.—Possible division forms of latent bodies of *T. rhodesiense* from heart and lungs of rats. Fig. 24 magnified 1,350 diameters. These forms are rare.

Figs. 25, 26.—Pyriform latent bodies, each with nucleus and blepharoplast. Fig. 25, of *T. rhodesiense* from blood of guinea-pig; fig. 26, of *T. gambiense* from blood of rat.

PLATE XXVII



LATENT & OTHER FORMS OF
TRYPANOSOMA GAMBIENSE & T. RHODESIENSE.

Figs. 27, 28.—Pyriform bodies of *T. rhodesiense* and *T. gambiense* (fig. 28) from peripheral blood of guinea-pig. In fig. 28 the flagellum is shown beginning to develop.

Figs. 29, 30.—Two pyriform non-flagellate forms from spleen of rat.

Fig. 31.—Round form of *T. gambiense*, with nucleus, blepharoplast and short flagellum along the edge of the body; from peripheral blood of rat.

Figs. 32-40.—Crithidia-like forms. Fig. 32, of *T. gambiense* from blood of rat; figs. 33-35, of *T. rhodesiense* from lung (fig. 33 and peripheral blood of rat; figs. 36-38, of *T. gambiense* from blood of guinea-pigs; figs. 39-40, of *T. rhodesiense* from blood of guinea-pigs. In fig. 37 note the basal granule ('blepharoplast' of Minchin) at the root of the flagellum, forming the centrosome of the kinetonucleus.

Fig. 41 represents an almost trypaniform stage of *T. gambiense* from blood of guinea-pig. Note the peculiar position of the blepharoplast (kinetonucleus).

Fig. 42 represents a stout trypaniform parasite from heart blood of rat.

Fig. 43 shows peculiar, large rounding form (from blood of a rat), exhibiting signs of division of its nucleus and blepharoplast.

Figs. 44, 45.—Slightly irregular parasites, very pale staining and vacuolated. Degenerating, as seen in detachment of flagellum, etc.

Figs. 46-49.—Degenerating trypanosomes, exhibiting chromatolysis, all from lungs of rats. Fig. 46 of *T. gambiense*, magnified 1,350.

Fig. 50.—Degenerating *Trypanosoma gambiense*, showing vacuolation, pale-staining cytoplasm and beginning of disintegration.

EXPERIMENTS ON THE TREATMENT OF ANIMALS INFECTED WITH TRYPANOSOMES BY MEANS OF ATOXYL, VACCINES, COLD, X-RAYS AND LEUCOCYTIC EXTRACT; ENUMERATIVE METHODS EMPLOYED*

BY
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AND
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(*Received for publication* 15 *October*, 1910)

These experiments were conducted with funds given by Sir Edwin Durning-Lawrence, Bart., for the purpose of testing the effect of cold on disease, and were suggested by one of us (R.R.) as a part of the studies made in connection with his case of sleeping sickness, as reported upon in an accompanying paper by him and Dr. David Thomson. Although many researches have been made on the effect of atoxyl and other drugs, we believe that these are the first in which that effect has been measured by regular daily counts of the parasites by measured thick-film methods.

I. ATOXYL

(*a*) *Small doses.*

From a study of the patient, W.A., at the Royal Southern Hospital by Major R. Ross and D. Thomson it will be noted that atoxyl failed to be of any marked benefit to the patient, and in the doses administered there was no trypanocidal action detected. It was necessary, therefore, to begin a series of experiments on animals inoculated with *T. rhodesiense* (Stephens and Fantham †),

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[*] An abstract of this paper was read before the Royal Society on December 8th, 1910, and published in Proc. Roy. Soc., B, Vol. LXXXIII, pp. 227-234 (1911).

[†] Proc. Roy. Soc., Series B, Vol LXXXIII, pp. 28-33 (1910), and Ann. Trop Med and Parasit., Vol. IV, pp. 343-351.

and to try the effect of small doses of atoxyl in these animals with a view to determine the effect of that drug on this particular trypanosome which, as will be seen from the accompanying paper on 'Enumerative Methods in Untreated Animals' (H. B. Fantham and J. G. Thomson[*]), was of extraordinary virulence. Various doses of atoxyl are recommended in treatment of human trypanosomiasis, and we find that the patient, W. A., in the Royal Southern Hospital, received four grains as a maximum dose. It was impossible in the case of this patient to push the drug further as he quickly showed signs of the toxic action of the drug. Louis Martin and Henri Darré[†], in an article on the treatment of sleeping sickness at the Pasteur Hospital, believe that atoxyl can cure light forms of the disease, and even severe forms, but that it is often necessary to give large doses, viz., one gram, which imperil vision. These authors however, still admit that permanent cure is a doubtful matter.

In the experiments made by us we attempted, as far as possible, to administer a therapeutic dose of atoxyl. We first sub-inoculated four rats subcutaneously with *T. rhodesiense*. The lightest in weight, Rat 34, weighed 139 grams, and the heaviest weighed 256 grams, namely, Rat 37.

	Colour	Weight	Inoculation period	Duration of life	
Rat 34 ...	Piebald	139 grams	5 days	27 days	Animal House
Rat 35 ...	Piebald	145 grams	5 days	26 days	Cold Chamber
Rat 36 ...	White	251 grams	6 days	27 days	Cold Chamber
Rat 37 ...	White	256 grams	5 days	8 days	Animal House

All the four rats were adults. Two were piebald and two white, and all received, subcutaneously, the same dose of trypanosomes, namely, 200,000. Two of them, 35 and 36, were placed in the cold chamber, where the lowest temperature recorded was 20° F., and two were placed in the animal house. All these animals received atoxyl in solution subcutaneously in small repeated doses, which varied from $\frac{1}{80}$ to $\frac{1}{20}$ of a grain. Take the body weight of a man as being

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[*] Proc. Roy. Soc., B. Vol. LXXXIII, 1911, pp. 206-211, and Ann. Trop. Med and Parasit Vol. IV, pp. 417-463.

[†] Bulletin of Sleeping Sickness, Vol. II, No. 18, 1910.

70 kilograms and the weight of our four rats as varying between 139 grams and 256 grams, and we find that the doses we administered to the rats, when calculated to the body weight of a man would represent doses varying between 4·55 grains and 25·14 grains. Rats 34 and 35, being the lighter rats, having received the same doses as Rats 36 and 37, therefore received a relatively larger dose when we consider the body weight. We now append tables of numbers of trypanosomes per c.mm. of peripheral blood and the doses of atoxyl given to Rats 34, 35, 36 and 37, and we also give graphs of the Rats 34, 35 and 36, which show the variations of the numbers of parasites in the peripheral blood.

RAT 34.—Piebald, weight 139 grams. Treated in Animal House with small doses of atoxyl. Inoculation dose: 200,000 *T. rhodesiense*

Day ...	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood						1,392	26,960
Atoxyl in grains				—	—	—	$\frac{1}{80}$
Day . .	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	40,460	8,118	1,440	10,500	5,888	101,200	96,800
Atoxyl in grains	$\frac{1}{60}$		$\frac{1}{60}$	—		$\frac{1}{80}$	—
Day . ..	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood . ..	73,920	4,000	20,496	5,796	12,696	3,864	19,136
Atoxyl in grains	$\frac{1}{30}$		$\frac{1}{30}$		$\frac{1}{20}$	—	$\frac{1}{30}$
Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm. of blood . ..	24,000	73,920	48,000	42,320	168,960	242,880	—
Atoxyl in grains .		$\frac{1}{30}$	$\frac{1}{30}$		—	$\frac{1}{30}$	—

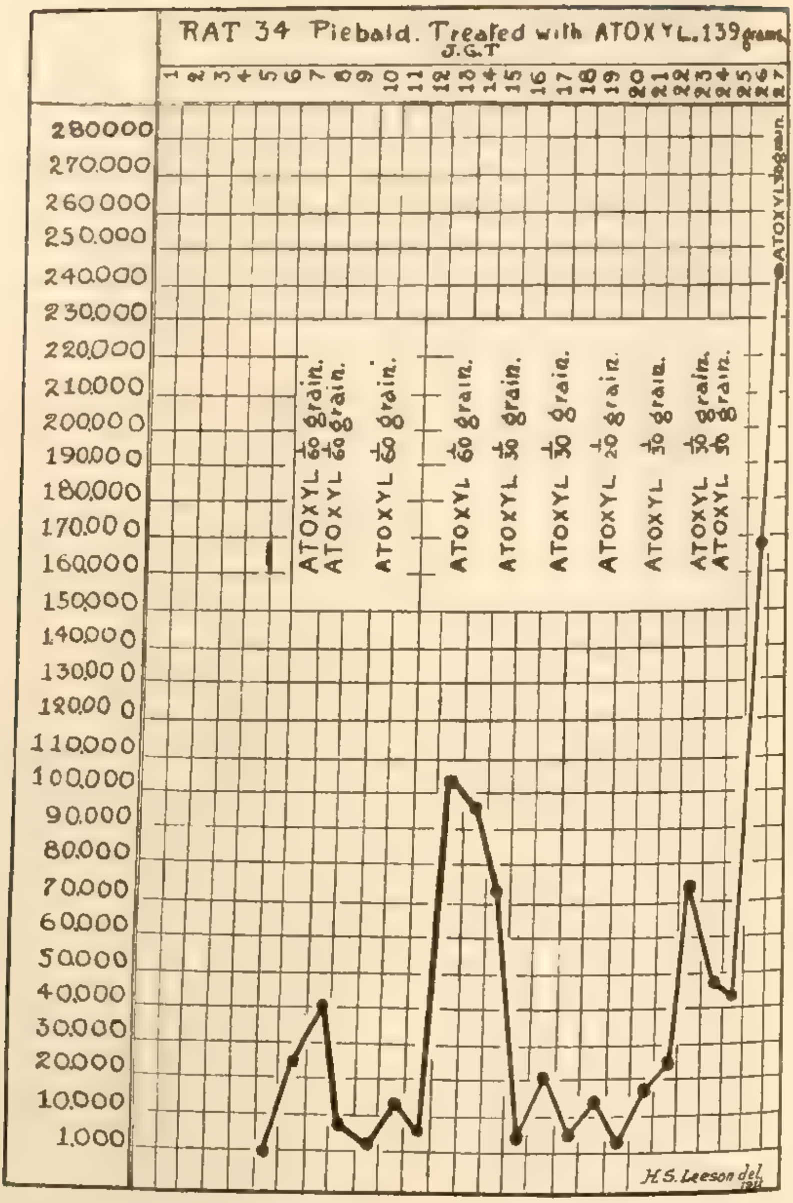

CHART 1.—Rat 34, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood

RAT 35.—Piebald Adult Rat, weight 145 grams. Treated in Cold Chamber with small doses of atoxyl. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—				·2,064	25,112
Atoxyl in grains	—	—		—		—	$\frac{1}{60}$

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	10,400	27,968	34,320	1,520	72,128	73.920	154,000
Atoxyl in grains	$\frac{1}{60}$	—	$\frac{1}{60}$	—	—	$\frac{1}{[illegible]}$	—

Day .	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	80,560	230,400	221,600	18.800	331,296	384,384	338,688
Atoxyl in grains	$\frac{1}{30}$	—	$\frac{1}{30}$		$\frac{1}{30}$		$\frac{1}{30}$

Day	22	23	24	25	26	—	
Number of Trypanosomes per c.mm. of blood ..	390,000	550,000	447,680	383,040	570,000	—	—
Atoxyl in grains	—	$\frac{1}{30}$	$\frac{1}{30}$	—		—	—

RAT 36.—White Adult, weight 251 grams. Treated in Cold Chamber with small doses of atoxyl. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	—	192
Atoxyl in grains	—	—	—	—	—	—	—

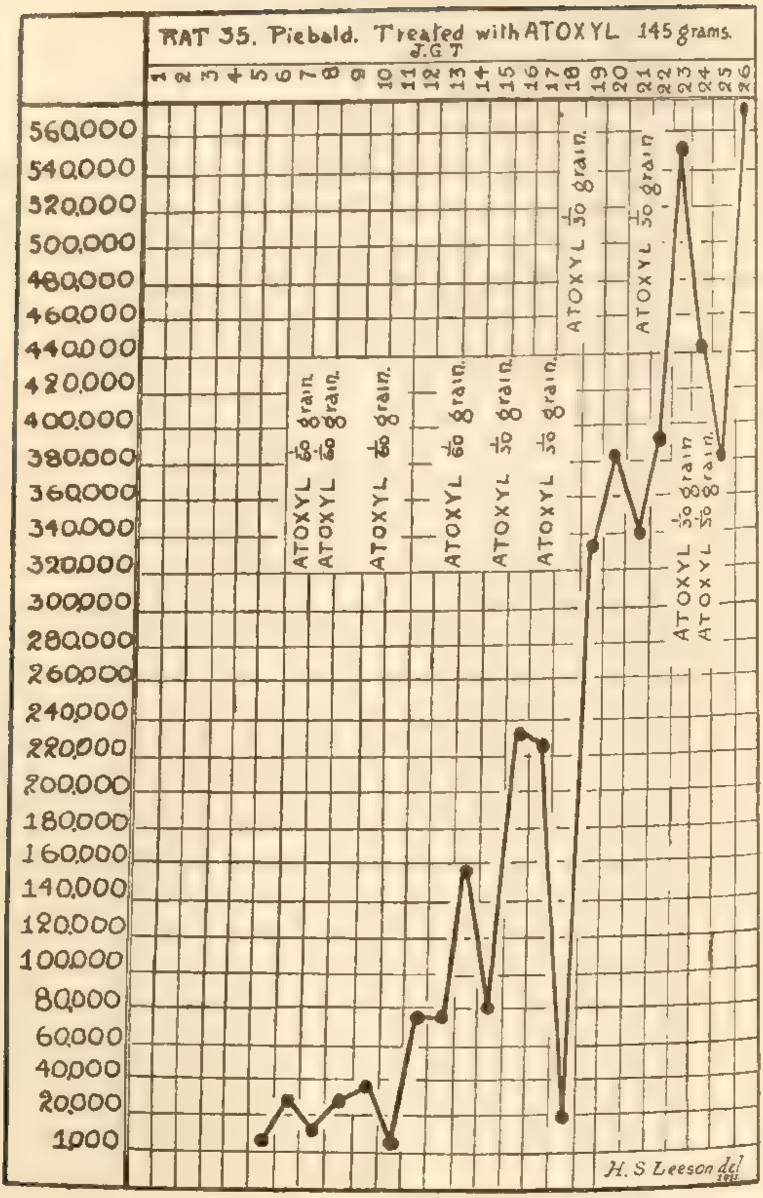

CHART 3.—Rat 35, inoculated with *T. rhodesiense*. Continuous line indicates the graph of [illegible] trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

Rat 36—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	34,356	70,560	75,600	80,640	168,544	66,528	125,400
Atoxyl in grains	$\frac{1}{60}$	$\frac{1}{60}$		—		$\frac{1}{60}$	-

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	300,000	241,000	336,000	316,800	404,800	337,920	363,000
Atoxyl in grains	$\frac{1}{10}$	—	-		$\frac{1}{30}$	-	$\frac{1}{30}$

Day	22	23	24	25	26	27	
Number of Trypanosomes per c.mm. of blood	[illegible]	281,600	138,000	530,000	483,840	500,000	
Atoxyl in grains	—	[illegible]	$\frac{1}{30}$	-	—	$\frac{1}{30}$	—

RAT 37.—White, weight 256 grams, Adult. Treated in Animal House with small doses of atoxyl. Animal developed Pneumonia

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm. of blood ..			-	—		4	2,004	32,640
Atoxyl in grains	-			—	—	-	$\frac{1}{60}$	$\frac{1}{60}$

If now we examine table and chart of Rat 34 we find that the period between the crests of the waves representing the numbers of trypanosomes was certainly longer than in those of the untreated animals inoculated with *T. rhodesiense* (*vide* Paper on 'Enumerative Methods in Untreated Animals,' H. B. Fantham and J. G. Thomson*). We find there were two high crests in the graph of this animal corresponding to five days and ten days. The weight of this animal was 139 grams, and thus the doses administered were

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* Proc. Roy. Soc., B, Vol. LXXXIII, 1911, pp. 206-211, and Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463.

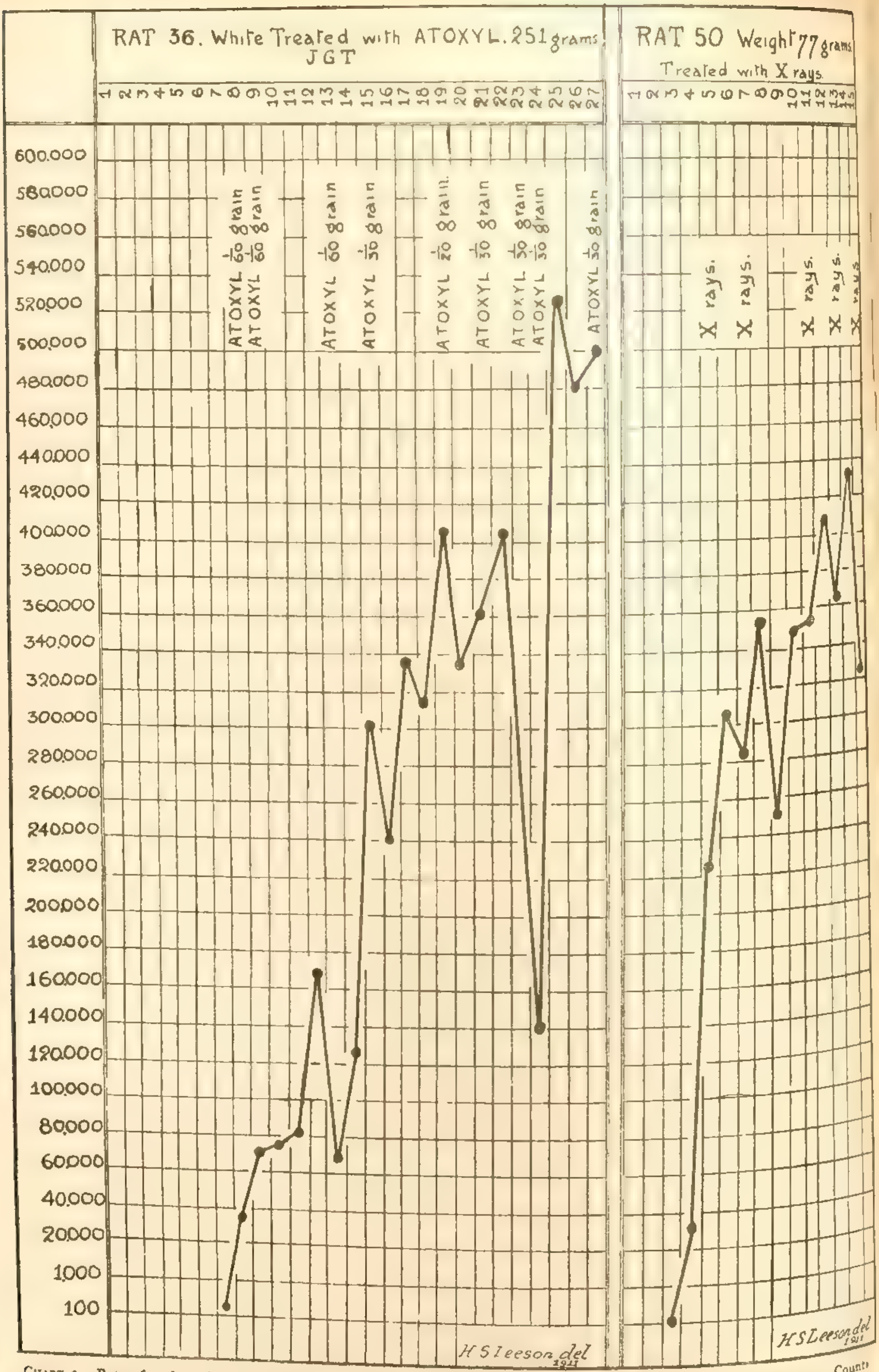

CHART 3.—Rats 36 and 50, inoculated with *T. rhodesiense.* Continuous line indicates the graph of the trypanosomes Counts made every 24 hours per c.mm. of peripheral blood.

relatively large. The doses given may have had a slight trypanocidal action, but from the appearances of the smears we do not think there was any marked degeneration, and many trypanosomes were found actively dividing. The doses given to this animal would, if calculated to the body weight of a man of 70 kilograms, represent approximately 8·38 grains, 16·76 grains, and 25·14 grains. The lengthened period between the crests of the waves may have been caused by natural body resistance being raised. This animal lived twenty-seven days, whereas the longest period of life recorded in our controls, sub-inoculated with the same strain, was eighteen days in an animal weighing 48 grams.

If we examine now Rat 35, which was treated with similar doses of atoxyl, and kept in the cold chamber, we find quite a different character in the graph representing the numbers of trypanosomes in the peripheral blood. This animal was piebald in colour and weighed 145 grams, that is to say, it was only slightly heavier than Rat 34. This means that the doses given when calculated to the body weight of a man were smaller than those given to Rat 34. Here the doses would represent in man 8·04 grains, 16·08 grains and 24·12 grains. In spite of these doses the trypanosomes in the peripheral circulation rapidly increased in numbers with a staircase effect, and on the day before death reached the enormous number of 570,000 per c.mm. of blood. This animal lived twenty-six days, and received during that time ten doses of atoxyl. It cannot be said in this case that the atoxyl in the doses given had any trypanocidal action, but, again, the life of the animal was undoubtedly prolonged by the drug. The numbers of division forms seen in the smears of this animal showed that the proliferation of the trypanosomes was very active, and we also noted that the proportion of short, stumpy forms was also markedly increased.

Rat 36, white in colour, and treated in the cold chamber with small doses of atoxyl, shows a chart which in many respects simulates the chart of Rat 35. Here, again, we note the staircase rise of the trypanosomes in spite of continued treatment with small doses of atoxyl. This animal being heavier than Rats 34 and 35, received, therefore, doses which, when calculated to the body weight of a man, were relatively small, namely, 4·64 grains, 9·28 grains and 13·92 grains.

In this animal all the smears gave evidence of the trypanosomes being abnormally stimulated to division. Many of the trypanosomes were seen to be dividing into four, and the numbers before death reached 530,000 per c.mm. of blood.

This animal lived twenty-seven days. If we do not attribute the prolonged life of these three rats to the trypanocidal action of the atoxyl we must endeavour to explain it in another manner.

Arsenic in small doses affects metabolism and improves the general condition of the body. In small doses we find in man, both in health and disease, that the strength, weight and appetite are improved, and it is quite to be expected, therefore, that small repeated doses of atoxyl in rats would tend to raise the natural body resistance. In referring to Cushny's *Pharmacology* we find that Gies treated young rabbits with arsenic, and found that those treated weighed more, had larger bones, and more developed muscles than the untreated controls. It was also found that a pregnant female rabbit treated with arsenic gave birth to young rabbits of abnormal size. That arsenic, therefore, in small doses profoundly affects metabolism and improves the general condition we have good evidence, and this might possibly explain the lengthened life of the animals treated with atoxyl in small doses. In the same way the lengthened period between the crests of the waves in Rat 34 may possibly have been due to increased resistance of the body of the host rather than to a direct action of the drug administered upon the parasites.

In spite of the fact that the animals' lives were prolonged we had the trypanosomes increase steadily in numbers, and this was very marked in the cases of Rats 35 and 36. The numbers of trypanosomes in the peripheral blood remained consistently high, and the smears showed very active divisions of parasites. This can be attributed to the action of the atoxyl in small doses. We have already referred to the general action of arsenic in small therapeutic doses. The drug seems to act as a tonic to the body in health as well as disease, and must, therefore, improve the condition of the cells of the whole body. Stockman and Greig* found the bone marrow in a state of unusual activity when arsenic was administered in small doses, and there were greater numbers of red blood corpuscles.

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* See Cushny's 'Pharmacology' on actions of arsenic.

Is it not possible, therefore, that atoxyl in small doses, such as given to these rats acted not only upon the cells of the body of the host, but on the trypanosomes themselves, and acted as a tonic to both, so stimulating increased division of the parasites? This would explain both the extraordinary division forms of the trypanosomes and also the prolonged life of the host without attributing to the atoxyl any trypanocidal action whatever.

It must be remembered, however, that we are working with a specially virulent strain of trypanosomes, and that, therefore, it may be peculiarly resistant to the action of atoxyl, which drug in many cases of human sleeping sickness seems undoubtedly to have a favourable influence on the disease.

Rats 35 and 36 were treated in the cold chamber and received atoxyl as well. In our experience, however, we find that although the cold tends to prolong the life of animals infected with trypanosomiasis to a slight extent it has not, as a rule, any marked effect upon the numbers of trypanosomes in the peripheral circulation, and thus we attribute the numerous division forms to the atoxyl.

(*b*) *Large doses of Atoxyl.*

We now tried the effect of a large dose of atoxyl on three rats, all heavily infected with *T. rhodesiense*.

Here we were guided in our experiments by the work of Thomas and Breinl[*], who pointed out that if a cure was to be expected in animals a large dose of atoxyl was necessary.

Rat 38. Piebald, weight 2[illegible]2 grams. (See Chart.) Dose of inoculation: 200,000 Trypanosomes. *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. ...						202	6,162
Atoxyl 5 per cent. solution ...	—						
Leucocytes							

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[*] Memoir XVI, Trypanosomiasis research, pp. 52-57, Sch. of Trop. Med., Liverpool, October, 1905.

Rat 38—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	28,[illegible]	86,000	43,[illegible]	21,736	0		
Atoxyl 5 per cent. solution ...				0·5 c.c.			
Leucocytes			..	16,368	24,720	63,[illegible]	4[illegible]

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.		1,600	18,[illegible]	32,[illegible]	12,[illegible]	16,[illegible]	[illegible]
Atoxyl 5 per cent. solution ...						0·5 c.c.	—
Leucocytes	22,880	—		—	—	12,360	24,000

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	320	4,51[illegible]	12,[illegible]	20,[illegible]	60,000	23,600	22,3[illegible]
Atoxyl 5 per cent. solution ...	—						—
Leucocytes	45,000	—					-

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	7,5[illegible]	760	84	320	2,6[illegible]	4,880	15,6[illegible]
Atoxyl 5 per cent. solution ...							
Leucocytes							

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	23,000	79,488	123,600	193,000	177,744	350,000	144,000
Atoxyl 5 per cent. solution ...							—
Leucocytes	-	33,120	16,000	—	40,320	10,400	4,248

Rat 38—continued.

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm. ...	317,856	218,880	231,840	211,520	112,640	190,520	664,608
Atoxyl 5 per cent. solution ..		—	—	—	–	–	—
Leucocytes	21,924	—	31,020	9,280	26,048	19,404	33,120

Day	50	51	—	-	–	—	-
Number of Trypanosomes per c.mm.	816,960	1,520,000	-		—	—	-
Atoxyl 5 per cent. solution ...		—	—	–	.	-	—
Leucocytes	63,480	-	—	—	–	—	-

The above animal, Rat 38, lived fifty-one days. The control of this animal weighing 238 grams lived eighteen days, and the day before death had 350,000 trypanosomes per c.mm. Rat 38 received two doses of a 5 per cent. solution of atoxyl (0·5 c.c.) subcutaneously, and it will be noted that this dose calculated to the body weight of a man of 70 kilograms would be 130 grains approximately. It is of interest to note that the leucocytes rose in Rat 38 on the thirteenth day to 63,000 per c.mm.

Two other rats infected with the same strain received doses of 0·5 c.c. of a 5 per cent. solution of atoxyl.

Rat 40, white, weight = 129 grams.
Rat 41, white, weight = 113 grams.

Rat 40 had 4,800 trypanosomes in the cubic millimetre before injection, and next day we found 89 per c.mm., i.e., twenty-four hours after injection of atoxyl, and in forty-eight hours there were no trypanosomes in the peripheral circulation.

Rat 41 had 7,056 trypanosomes per c.mm. before injection of atoxyl and next day two, presumably dead trypanosomes, were found in a thick film. This rat, however, succumbed to the toxic action of the drug the following day.

Atoxyl, therefore, in large doses is a trypanocide. Arsenic in so-called poisonous doses causes adverse conditions in cells, and

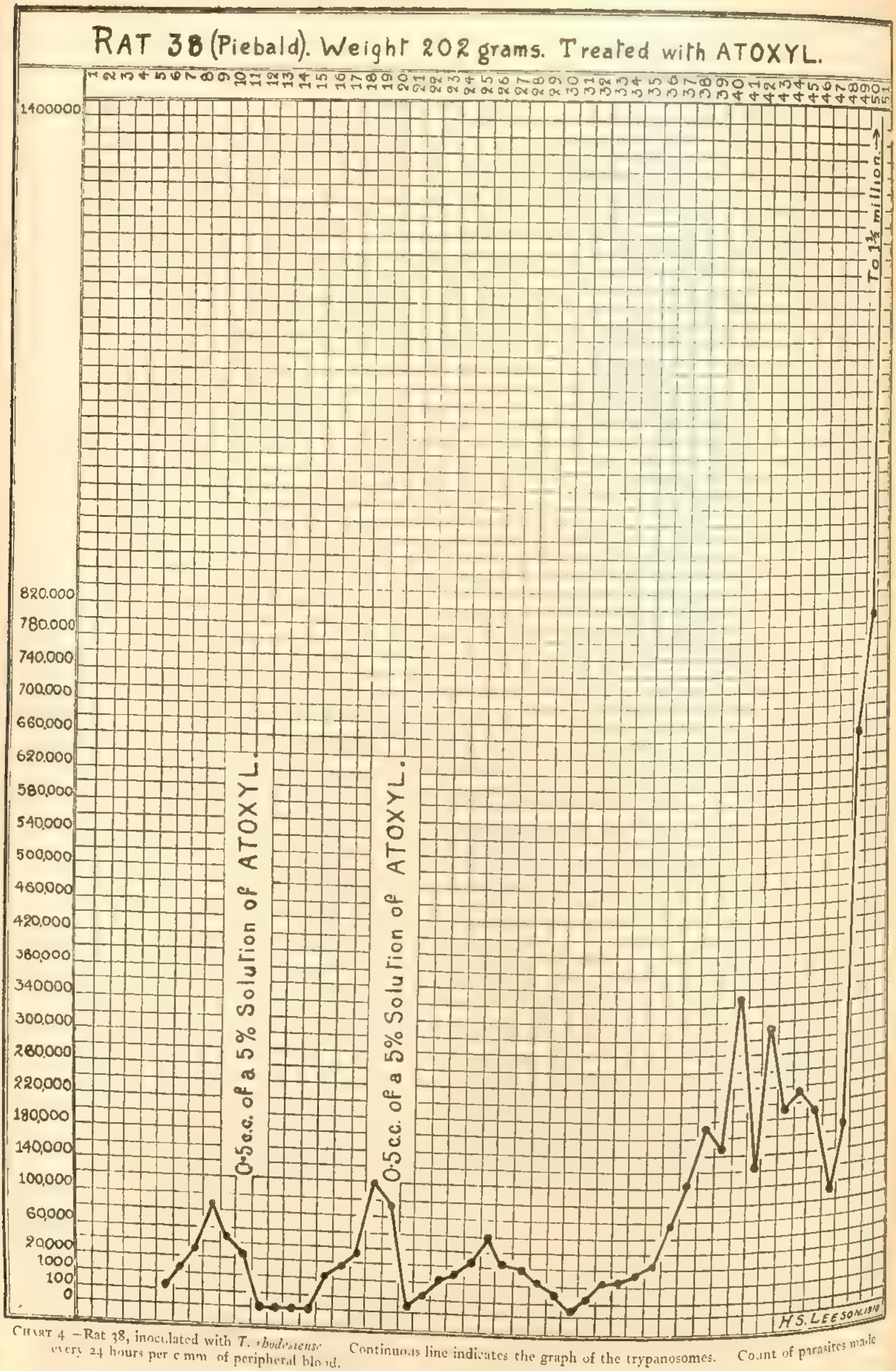

CHART 4 —Rat 38, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c mm of peripheral blood.

we get fatty degeneration, especially of the cells of the liver and the epithelial cells of the stomach and intestines, and to a lesser extent in the kidneys and other organs in the body. If, therefore, we give atoxyl in a poisonous dose to an animal we kill not only the trypanosome cells, but also the body cells.

Unfortunately, in this strain of trypanosomes, even after the large dose of atoxyl given, the parasites appeared again in Rat 38 after an absence from the peripheral blood of four days. This is only to be explained by the resistant forms of Moore and Breinl (Ann. Trop. Med., I, page 457 (1907)) forming in the spleen and bone marrow, and these again flagellate out under favourable circumstances.

Thomas and Breinl pointed out that to obtain a cure we must approach as near as possible to the lethal dose.

A small dose of atoxyl repeated did not act as a trypanocide, but we would suggest prolonged the life of the animal by acting as a tonic.

A large dose of atoxyl, on the other hand, is certainly a trypanocide, and clears the peripheral circulation of the parasites, but in this strain, *T. rhodesiense*, which is a particularly virulent one, the trypanosomes were evidently able to resist the action of the atoxyl by encysting themselves in the spleen and bone marrow (Moore and Breinl*).

OUR CONCLUSIONS ARE BRIEFLY THESE:—

(1) Small doses of atoxyl, i.e., therapeutic doses in this strain, viz., *T. rhodesiense*, prolong the life of the host, probably by raising the body resistance.

(2) Small doses of atoxyl in this strain of trypanosomes actually stimulate division of the parasites.

(3) Large doses of atoxyl kill the parasites, but probably do not cure the animal owing to atoxyl-resistant bodies (Moore and Breinl) being formed in the spleen and bone marrow.

(4) As has been noted by other observers in virulent forms of this disease, the initial dose must be large if a cure is to be expected.

(5) Atoxyl is *not* a specific in *T. rhodesiense*, but would appear to be almost as toxic to the body cells as to the trypanosomes themselves.

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* Ann. Trop. Med. and Parasit., Vol. I, p. 457 (1907).

Treatment of T. rhodesiense in rats by subcutaneous injection of vaccines.

II. VACCINE TREATMENT

In view of the extensive development of vaccine treatment in disease, we have carried out some experiments in the treatment of trypanosomiasis. These experiments are few, and therefore inconclusive, but they have produced results of interest, and we are continuing the experiments in the hope of gaining further knowledge.

The vaccine used by us was not a true vaccine, because it contained serum, blood corpuscles, leucocytes, and the bodies of dead trypanosomes. It will thus be noted that we injected a rather complicated fluid, and that it was only a vaccine in the sense that it contained the bodies of dead trypanosomes.

The vaccine was prepared by searing the surface of the heart with a hot needle in order to get a sterile portion, and through this the blood was drawn into a sterile pipette and mixed with normal saline. This mixture was placed for half an hour in an incubator at 55° C., and finally a little trikresol was added. A count was made of the parasites in 1 c.mm. before they were subjected to heat and appropriate dilution was carried out with normal sterile salt solutions.

There are two distinct points at which we may prepare this *so-called* vaccine. We may prepare it at the height of the rise of the trypanosomes in the peripheral blood, or it may be prepared when the trypanosomes are few in number.

In the former case we have a condition in which the numbers of trypanosomes are great, and probably the anti-bodies in the serum few. In the latter case we have, on the other hand, a condition where the trypanosomes are few and the serum rich in anti-bodies.

We have conducted experiments and used a so-called vaccine prepared at both the above stages. The first so-called vaccine was rich in trypanosomes, and was, therefore, more of the nature of a true vaccine than the latter, which was what we might call a vaccine *plus* an anti-serum.

We first experimented with a vaccine obtained at the height of the rise. Here it was noted that after subcutaneous injection a rise in the number of trypanosomes took place. The fact was first noted by D. Thomson, while conducting similar work on the human case of sleeping sickness. This observer asserts that an injection of a so-called vaccine—which was simply rats' blood, sterilised and mixed with normal saline—rich in the dead bodies of trypanosomes, stimulated the trypanosomes in the peripheral blood of the patient to divide, and the explanation seems to be based on the hypothesis of Dr. H. C. Ross, who holds that extracts of dead animal tissues stimulate cell division*.

In our experiments with rats infected with *T. rhodesiense*, we without exception found that when the blood of an animal rich in trypanosomes was injected subcutaneously, a rise of trypanosomes occurred during the next twenty four hours. This rise was sometimes very sudden and exceedingly high, and if the dose was large and strong, the rise was several times so great as to cause the death of the animal. This is exactly in line with the treatment of disease with true bacterial vaccines, and here it is well known that if we treat say a mastitis with vaccine and give an excessive dose, we aggravate the condition. On the other hand, if a small dose was given to an infected rat, we got during the next twenty-four hours a rise, but not sufficient to kill the rat. This rise may well be represented as the negative phase, and a fall occurs usually in forty-eight to seventy-two hours, which may be considered as the positive phase.

We conclude, therefore, that the dose is important, i.e., it must not be too large, and secondly, we must at least allow an interval of one day before a second dose is administered.

In our first experiments we used six rats infected with *T. rhodesiense*, and vaccine was prepared from another rat, which was killed when the trypanosomes were at the height of a crest. We thus used a blood rich in trypanosomes, and relatively poor in anti-bodies. Three rats were kept as controls and three were treated. Two of those treated quickly succumbed, and we explain this by the fact that we gave two doses of vaccine without allowing an interval of at least twenty-four hours. We gave no time for a reaction to take place. The third rat, however, lived longer than the controls, and in this case, if we eliminate the fact that it may have been a more resistant animal, we had the life prolonged by the vaccine, which was given with an interval of one day between the doses.

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* Brit. Med. Journ., June 11, 1910.

We attempted the same line of treatment in two guinea-pigs, but here again, we cannot say that we prolonged life. We now attempted to obtain results with a blood prepared when the trypanosomes were low in the peripheral blood, i.e., we tried a condition in which one would expect to find anti-bodies, and in addition to this the blood was rich in leucocytes. The first injection, given on the eighth day, produced a fall in the trypanosomes and a rise in leucocytes which was very marked. The second injection was given on the thirteenth day, and again we obtained a fall (slight), but the leucocytes also fell. On the twentieth day we gave an injection of blood obtained from an animal when the trypanosomes were numerous; a very high rise took place and the animal died. This animal lived twenty-two days, i.e., we may conclude that we prolonged its life, and, indeed, the animal might have lived a longer period had we not on the twentieth day given too strong a dose of vaccine. We publish the chart of the last experiment (Rat 95), as it shows the rise and fall of the leucocytes in relation to the rise and fall of trypanosomes. It will be noted, if we refer to chart of Rat 95, that after inoculation a leucopenia takes place, and when trypanosomes first appear in the peripheral blood we have the leucocytes increasing. Again, it will be noted that when the trypanosomes are high in number that there tends to be a fall in the number of leucocytes, whereas when the trypanosomes are low in the peripheral blood there tends to be a marked leucocytosis. We have noticed this relation between leucocytes and trypanosomes in many cases.

We append tables of numbers of three untreated controls, viz., Rats 14, 15 and 16, and the tables of three rats treated with vaccine of a blood rich in trypanosomes, viz., Rats 42, 43 and 44, and, lastly, we append a chart of Rat 95 infected with *T. rhodesiense*. Here the first two doses of so-called vaccine were prepared from a rat when the trypanosomes were few in the peripheral blood, and we note that a fall in the numbers took place

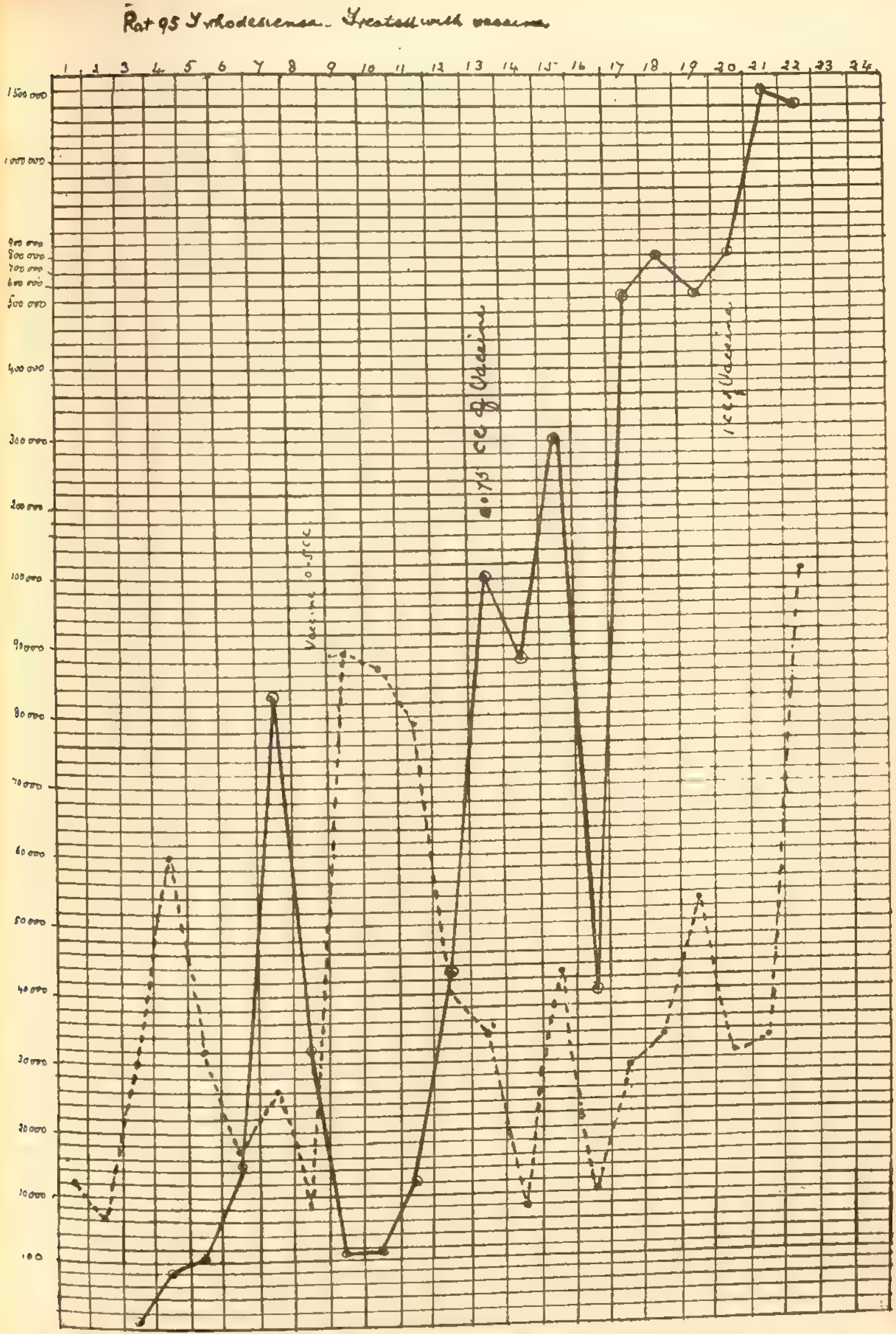

Chart 5 · Rat 95. ——— Continuous line indicates the trypanosome graph
- - - - - - - - Dotted line indicates the graph of leucocytes. Counts made per c.mm. of peripheral blood.

after these injections. The last injection of vaccine, given on the twentieth day, was prepared from a rat when the trypanosomes were very numerous, and we note that there was in the next twenty-four hours an enormous increase in the numbers of trypanosomes, from which the animals were evidently unable to recover.

CONTROLS—NO TREATMENT

RAT 14.—Piebald, weight 127 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	1,440	45,600	66,240	7,144
Leucocytes	—	—	—	—	26,080	36,784	59,648

Day	8	9	10	11	12		—
Number of Trypanosomes per c.mm.	50,232	77,440	250,000	464,640	500,000		
Leucocytes	—	—	—	—	—	—	—

RAT 15.—Piebald, weight 104 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	4,620	45,440

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	24,472	44,352	194,128	410,000	—	—	—

Rat 16.—White, weight 58 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.					104	[illegible]	[illegible]
Leucocytes		[illegible]	14,000	21,060	[illegible]		
Temp.		42	44	36		44	46

Day	8	9	10	11	12	13
Number of Trypanosomes per c.mm.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	No count
Leucocytes					8,416	
Temp.	34	[illegible]	[illegible]	34	—	[illegible]

RATS TREATED WITH VACCINE

Rat 42.—White, weight 121 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—					90	24,000
Vaccine							

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	2,376	[illegible]	9,044	35,200	60,480	32,000	123,200
Vaccine							

Day	15	16	17	18	19
Number of Trypanosomes per c.mm.	136,160	185,600	239,520	306,432	211,600
Vaccine	10,000,000	—	10,000,000	10,000,000	

Rat 42—continued.

Day	20	21		—	—	—	—
Number of Trypanosomes per c.mm. ...	225,872	211,600	—				
Vaccine ...			—	—	—	—	—

Rat 43.—White, weight 140 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6
Number of Trypanosomes per c.mm. ...	..		—	840	[illegible]	[illegible]
Vaccine					12,000,000	12,000,000

Day	7	8	9		—	
Number of Trypanosomes per c.mm. ...	144,000	310,000	5,292			
Vaccine			—			—

Rat 44.—Piebald, weight 237 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6
Number of Trypanosomes per c.mm.			65	35,296	42,320	248,960
Leucocytes		—	—	27,446	15,608	30,400
Vaccine		—	—		12,000,000	12,000,000

Day	7	8	9	10	11	—	—
Number of Trypanosomes per c.mm.	13,056	39,960	97,544	310,000	366,000	—	
Leucocytes	43,000						
Vaccine			—		—		—

III. TREATMENT IN THE COLD CHAMBER

The dimensions of the cold chamber are twelve feet long by seven feet wide by six and three-quarter feet high, and this is cooled by a refrigerator.

The lowest temperature reached in our experiments was 20° F. As the cooling apparatus was stopped during the night the temperature rose, and in the morning, before starting the machinery, reached 36 F. to 38 F., but this, of course, varied with the temperature of the atmosphere outside the chamber.

The humidity of the atmosphere in the chamber was low, and was found to vary between 50 and 60 per cent., but this also varied with the humidity outside, as the door had to be opened several times daily for the purposes of observation and to admit of feeding the animals.

For the above observations regarding humidity and temperature we are indebted to Major Williams, who compares the atmosphere of the cold chamber to that in the interior of Canada.

We were thus enabled to carry out treatment in a cold, dry atmosphere, and from personal experience we can testify to the invigorating feeling bestowed on us by a short sojourn in this chamber.

The animals in this chamber were active and took their food well, and, without doubt, seemed much better than their controls outside in the animal house.

The patient W.A., suffering from sleeping sickness contracted in Rhodesia, several times visited the cold chamber for treatment, but, unfortunately, at this time, no counts were made of the parasites in the peripheral circulation, but we have the evidence of the patient himself, who emphatically declared that he felt much better after being for some time in the cold chamber.

As the patient became worse, treatment had to be discontinued owing to the fact that the patient was considered too ill to travel from the Royal Southern Hospital to the University, and we had to resort to treatment in animals alone.

The animals used were guinea-pigs and rats, and we used two strains of trypanosomes—

(1) The *T. rhodesiense* obtained from patient W.A. (Ross and Thomson, 1910[*]), and

(2) An old laboratory strain of *T. gambiense*.

The essential differences between these two strains has been discussed by H. B. Fantham and J. G. Thomson in Paper on 'Enumerations in Untreated Animals suffering from Sleeping Sickness,' 1910[†].

We at first selected guinea pigs sub-inoculated with sleeping sickness, and from the tables we append it is evident that the animals were more resistant to the infection while living in the cold chamber.

T. gambiense. Old Laboratory Strain. Controls in Animal House

Animal	Incubation	Duration of life	Weight in grams
Guinea-pig 1[1]	4	28	495
Guinea-pig 2[1]	4	30	580
Guinea-pig 3[2]	4	105	534
Guinea-pig 4[2]	2	79	403

[1] Counts of these animals are not given.

[2] Daily counts of these animals are given in the paper on Enumerative Studies on Trypanosomes in Animals by Fantham and J. G. Thomson, where Guinea-pig 3 is numbered as 7 and Guinea-pig 4 is numbered as 6.

T. gambiense. Old Laboratory Strain. Treated in cold. Lowest Temp. 20° F.

Animal	Incubation	Duration of life	Weight in grams
Guinea-pig 5	19	102	648
Guinea-pig 6	6	93	562
Guinea-pig 7	16	74	623

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[*] Proc. Roy. Soc., B, Vol. LXXXIII, pp. 187-205 (1911)

[†] Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211 (1911), and Ann. Trop. Med. and Paras Vol. IV, pp. 417-463.

GUINEA-PIGS.—Sub-inoculated with *T. rhodesiense*

No.		Incubation	Duration of life	Weight in grams	
Guinea-pig 8	...	13 days	120 days	350	Treated in Cold Chamber
Guinea-pig 9[1]	...	6 days	79 days	346	Treated in Animal House

[1] This is Guinea-pig 1 in paper on Enumerative Studies on Trypanosomes by Fantham and J. G. Thomson.

From the above tables it will be noted that the incubation period was delayed in all cases in the animals subjected to treatment in the cold chamber.

In the above tables Guinea-pigs 1, 2 and 3 were controls of Guinea-pigs 5 and 6.

Guinea-pig 4 was control of Guinea-pig 7, and Guinea-pig 9 was control of Guinea-pig 8.

The average incubation period of the five controls in the animal house was four days, whereas the average incubation period of the four animals treated in the cold chamber was 13½ days.

The average duration of life in the controls was 64⅕ days, whereas in the animals treated in the cold chamber the average life was 97¼ days.

In addition to this the animals in the cold were livelier, and took their food better than those in the animal house.

We can, therefore, conclude that in guinea-pigs sub-inoculated with both strains of sleeping sickness we had the incubation period delayed in the cold, and also the life of the animals prolonged when treated by cold.

We now experimented with rats. Here, again, we sub-inoculated the animals with the *T. rhodesiense* from the patient W.A., and we also sub-inoculated a series of rats with the old laboratory strain of *T. gambiense*.

CONTROLS. Old Laboratory Strain. *T. gambiense*

Number Piebald Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 23	173	6	19	2,000,000	8
Rat 27 ...	113	5	15	60,000	7
Rat 28	101	5	17	60,000	3

Cold Chamber. Old Laboratory Strain. *T. gambiense*

Number Piebald Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 45	270	7	12	[illegible]	[illegible]
Rat 46 ...	113	6	26	[illegible]	Fall in first 24 hours

Animal House. *T. rhodesiense*

Number White Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 17	[illegible]	4	[illegible]	38,000	A fall
Rat 16	58	4	13	200,000	8 divisions

Cold Chamber. *T. rhodesiense*

Number White Rat	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 47	90	6	11	[illegible]	4 divisions

It will be noted from the above that the average incubation period of those in the cold chamber was 6·3 days, whereas the incubation period of the control was 4·8 days.

The average life in the cold was 16·3 days, and the average of those in the animal house was 14·2 days.

Rat 23 is the control of Rat 45. Rats 27 and 28 are controls of Rat 46. Rat 17 is the control of Rat 47.

We now append a table of the daily counts of the trypanosomes in the peripheral blood of animals treated in the cold. These counts were made every twenty-four hours. The incubation period is stated in days, and we define it as the period when trypanosomes are first found in one quarter cubic millimetre of peripheral blood.

The temperature in the tables is recorded according to the haematothermic scale of one of us (R.R.), where temp. = (F. − 95) × 10, F. being the temperature in degrees Fahrenheit recorded by a clinical thermometer. Temperature was taken in all cases per rectum. The counts of Guinea-pigs 1 and 2 are not given. For the counts of Guinea-pigs 3 and 4 see paper on 'Enumerative Studies on Trypanosomes,' by Fantham and J. G. Thomson, where Guinea-pig 3 is numbered as 7, and Guinea-pig 4 is numbered as 6.

Guinea-pig 5.—Cold Chamber. *T. gambiense.* Incubation period 19 days. Duration of life 102 days

Day	20	21	22	23	24	25	26	27
Number of Trypanosomes per c.mm.	4	16	53	28	12	24	32	40
Temp.	55	82	82	80	80		83	95
Weight in grams		—	648					

Day	28	29	30	31	32	33	34
Number of Trypanosomes per c.mm.	92	1,060	2,208	5,568	7,108	10,476	4,806
Temp.	86	82	80	74	—	86	87
Weight in grams		603	–	—	-	-	-

Day	35	36	37	38	39	40	41
Number of Trypanosomes per c.mm.	4,192	160	408	200		300	684
Temp.	84	81	90	80		82	80
Weight in grams	—	608	—	—			-

Day	42	43	44	45	46	47	48
Number of Trypanosomes per c.mm.	—	-	1,040	1,520	—		2.880
Temp.	-	-	88			-	-
Weight in grams			-				

Guinea-pig 5—continued.

Day	49	50	51	52	53	54	55
Number of Trypanosomes per c.mm.	5,344	—	—	21,252	—	19,136	9,700
Temp.	80	—	—	86		82	—
Weight in grams		—	—	—	—	—	—

GUINEA-PIG 6.—Cold Chamber. *T. gambiense.* Incubation period six days. Duration of life 93 days. Killed accidentally by CO_2 anaesthesia

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c mm.	—	—	—	—	—	—	Tryps. present
Temp.	—	—	—	—	—	—	—
Weight in grams		—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams .	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	—	—	—	—	7,360	18,240	12,3[illegible]
Temp.	—	—	—	—	—	65	82
Weight in grams .. .	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	17,304	18,384	29,348	9,200	4,032	3,488	2,640
Temp.	90	86	93	96	94	92	86
Weight in grams	562	—	—	—	—		
Leucocytes	—	—	31,096	13,000	—		—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	2,880	880	52	144	416	1,716	3,526
Temp.	94	88	—	91	97	88	86
Weight in grams		—	—	—	—		515
Leucocytes			—	—	—		—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	4,366	10,504	—	15,420	13,380	—	—
Temp.	86	80	—	78	75	—	—
Weight in grams	—			—	—	—	—
Leucocytes	—	—				—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	840	840			2,016	4,620	
Temp.	80		—		—	78	—
Weight in grams			—	—	—		
Leucocytes		—		—	—	—	—

Day	50	51	52	53	54	—	—
Number of Trypanosomes per c.mm.	—	24,128	—	66,700	57,120	—	—
Temp.		74	—	82	—		—
Weight in grams	—	—	—	—		—	
Leucocytes	—	9,648		21,460	10,640	—	—

GUINEA-PIG 7.—Weight 623 grams. Cold Chamber. Dose of inoculation: 4,000,000. *T. gambiense*

Day ...	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. ...					-	-	
Leucocytes ...		—	48,000	—	—		
Temp. ...	76	88	96	—	86	80	9
Weight in grams	623					-	594

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. ...					—		
Leucocytes	—	—	—	—	—	—	-
Temp.	84	90	82	–	82	80	80
Weight in grams	-		—	-	—	-	-

Day ...	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	—		40	0	156	176	420
Leucocytes	-		—	—	—	—	-
Temp.	80	80	79		85	80	78
Weight in grams	570					—	–

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	408	600	2,688	2,800	16,640	3,584	2,688
Leucocytes	—	—	—	—	–	—	—
Temp.	82	82	80	–	–	74	88
Weight in grams	—	—	—	–	-	—	—

Guinea-pig 7—continued.

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	600	2,448	11,852	13,104	15,708	14,900	9,680
Leucocytes	—	—	5,244	13,000	47,256	27,800	31,328
Temp.	84	—	86	—	80	74	84
Weight in grams	533	—	—	—	—	—	-

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	10,480	22,720	17,572	39,672	42,240	30,000	46,240
Leucocytes	24,160	15,920	33,396	15,200	—	39,672	-
Temp.	75	78	74	—	70	76	74
Weight in grams	—				—	—	-

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	145,000	108,000	36,000	46,880	39,700	55,524	68,040
Leucocytes	13,632	23,472	—	33,920	17,640	11,424	11,772
Temp.	82	53	86		70	70	82
Weight in grams .	608	—	—	—	—	—	596

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	55,680	93,280	43,200	65,668	66,176	19,440	46,400
Leucocytes	4,000	8,500	—	16,588	—	-	
Temp.	82	80	82		78	80	78
Weight in grams	-				—	—	

Day	57	58	59	60	61	62	63
Number of Trypanosomes per c.mm.	52,376	70,056	78,800	44,800	21,160	37,296	62,160
Leucocytes . . .				—	—	-	
Temp.	50	80	81	-	-	95	85
Weight in grams	581					—	

Guinea-pig 7—continued.

Day	64	65	66	67	68	69	70
Number of Trypanosomes per c.mm.	—	65,296	56,800	41,920	46,720	69,840	36,640
Leucocytes		—	—	—	—	—	—
Temp.	90	—	—	—	78	—	—
Weight in grams	622	—	—	—	—	—	—

Day	71	72	73	74	75	—	
Number of Trypanosomes per c.mm.	70,664	64,584	21,672	6,880	Dead	—	—
Leucocytes		—	—			—	
Temp.	—		—	—	—	—	
Weight in grams	—	—	—	—	—	—	

Guinea-pig 8.—Cold Chamber. Weight 350 grams. Dose of inoculation: 500,000 *T. rhodesiense.* Incubation period 13 days. Duration of life 120 days.

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.		—		—	—	—	-
Leucocytes	—	35,000	—	18,304	29,456	32,208	10,208
Temp.	-	78	82	80	—	72	8
Weight in grams	-	350				--	-

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	-	-	—	8
Leucocytes	9.408	3,584	22,512	10,920	12,012	32,256	18,64
Temp.	83	68	80	76	-	74	82
Weight in grams	—	—	—	-	—	—	—

Guinea-pig 8—continued.

Day ...	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. ...	2	8	4	12	16	40	8
Leucocytes	—	14,352	—	—	—	—	—
Temp.	80	82	76	77		70	76
Weight in grams	—	369	—	—		—	—

Day ...	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	16	360	612	2,352	4,060	356	480
Leucocytes ...	—	11,760	21,692	—	30,740		—
Temp. ...	84	80	84	92		86	84
Weight in grams		387				—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	6,440	6,720	3,192	4,320	6,216	13,376	9,760
Leucocytes ...			—	—		—	—
Temp.	86	80	81	79	—	—	92
Weight in grams	—	410	—		—		

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm. ...	17,432	10,000	5,760	7,360	7,920	12,800	4,000
Leucocytes	—		—	—			
Temp.	82	83	—	—		87	
Weight in grams	—	443		—	—		—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	7,632	2,784	40,152	9,240	8,064	10,184	14,240
Leucocytes	—	—	—	—	—	—	—
Temp.			—	—	—	—	—
Weight in grams	—	—				—	

Guinea-pig 8—continued.

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	2,000	5,376	8,800	2,800	—	15,840	11,264
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams ...	—	—	—	—	—	—	—

CRYOTHERAPY EXPERIMENTS

Rat 23.—Piebald adult, weight 173 grams. Control in Animal House. Dose of inoculation: 2,000,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	120
Temp.	55	40	50	50	44	34	51
Leucocytes	-	—	29,000	—	—		—
Weight in grams	173	—	—	—	—	—	-

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	48,000	69,728	125,860	19,764	600	9,280	60,712
Temp.	45	65	50	—	50	66	38
Leucocytes	—	—	-	17,712	—	—	—
Weight in grams	167		—	—	—	-	

Day	15	16	17	18	19	—	—
Number of Trypanosomes per c.mm.	130,000	142,000	100,000	250,000	164,000	-	—
Temp.	34	40	55		11	-	—
Leucocytes					--	-	—
Weight in grams	160	-	—	—			—

Rat 27.—White adult, weight 113 grams. Control in Animal House. Inoculation dose: 60,000 *T. gambiense*

Day ...	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	120	14,310
Temp.	61	49	32	12	44		5
Leucocytes			—	—			—
Weight in grams	113		112				—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	101,740	146,880	69,400	23,276	55,224	67,096	57,552
Temp.	40	44	36	36	35	—	—
Leucocytes				—	—	—	—
Weight in grams						—	—

Day	15	
Number of Trypanosomes per c.mm.	6,120	Dead
Temp.		
Leucocytes		
Weight in grams	—	

Rat 28.—White adult, weight 101 grams. Control in Animal House. Inoculation dose: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.		—	—	—	—	256	2,808
Temp.	59	70	43	42	40	—	56
Weight in grams .	101		98	—	—	—	—

Rat 28—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	4,416	3,648	24,904	90,509	118,700	143,520	270,000
Temp.	44	42	34	26	24	-	40
Weight in grams ..	—	–	—		—	—	–

Day . .	15	16	17	18	—	—	–
Number of Trypanosomes per c.mm.	100,000	200,600	6,048	Dead	–	–	–
Temp.	78	42	-	—	—	—	—
Weight in grams	–	—	88	—	..	—	—

CRYOTHERAPY EXPERIMENTS

Rat 45.—Cold Chamber. Lowest temp. 20° F. Weight 270 grams. Dose of inoculation: 2,000,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	–
Temp.	54	70	60	-	56	52	60
Weight in grams	270	–		–		—	–
Leucocytes	—	—	45,000	—	—	—	-

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	92	24,012	480	430	1,100	—	–
Temp.	50	54	57		0	—	—
Weight in grams . ..	246	–	.	—	—	-	-
Leucocytes	- -	—	.		—	—	—

RAT 46.—Cold Chamber. Weight 113 grams. Dose of inoculation: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—		3,608
Temp.	60	60	50	78	60	—	63
Weight in grams ..	113	—	111	—	·	—	
Leucocytes	—	—	—	—			

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	3,456	2,352	8,640	2,400	7,100	3,104	13,024
Temp.	62	50	62	60	56		
Weight in grams			-	—	—		—
Leucocytes	—			—	--		

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	40,120	63,480	11,448	61,560	16,416	28,188	46,980
Temp.	40	52	50	55	—	—	70
Weight in grams	—	—	125	—		-	—
Leucocytes	—	14,260		12,236	26,432	22,736	23,160

Day	22	23	24	25	26	27	
Number of Trypanosomes per c.mm.	85,120	82,080	393,120	350,000	225,680	Dead	
Temp.	68	63	34	58	50		
Weight in grams	—		—	—	—	—	—
Leucocytes	8,136	20,832	23,000	25,688	—		—

RAT 17.—White, weight 62 grams. Animal House. Dose of inoculation: 38,400 *T. rhodesiense*

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	—		1,500	500	1,380	4,028
Leucocytes	—	—	-		38,700			—
Temp.	—	50	30	56	50	—		-

RAT 16.—White, weight 58 grams. Animal House. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.		-			104	37,000	30,24
Leucocytes		9,204	14,000	21,060	10,468	30,800	28,000
Temp.		42	44	36	—	44	46

Day	8	9	10	11	12	13	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	No count	
Leucocytes	18,584	27,360	16,340	38,124	8,416	—	
Temp.	34	52	20	34	-	18	—

RAT 47.—Cold Chamber. Weight 90 grams. Dose of inoculation: 38,400 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.		-	-	—	—	—	5,184
Leucocytes	—		--			—	-
Temp.	.	50	45	50	40	-	-

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	82,818	230,000	106,000	330,000	—	—	—
Leucocytes . . .		—	--	-	—	.	-
Temp.	40	66		36	—		

The conclusions to be drawn from the above experiments are therefore in favour of treatment in the cold. The resistance of the animal is evidently raised, for the incubation period is delayed, and the animal lives longer in the cold. In favour of the cold also, we have the evidence of the patient, W.A., who said he felt better when in the cold chamber. What the physiological action of cold is we are not prepared in our present state of knowledge to state, but we certainly think that a person warmly clad is beneficially acted upon by a cold, dry atmosphere, and we would suggest that patients suffering from sleeping sickness would be greatly benefited by a sojourn, say, in Canada or Switzerland. The cold seems to be a valuable therapeutic agent in treatment.

We must acknowledge the fact that much more must be done in this investigation before definite conclusions are drawn, but the results have been so encouraging that we think they ought to be made known at once.

IV. X-RAYS

A young piebald rat, weighing 77 grams was inoculated with the Rhodesian strain of trypanosomes.

Here we have to thank Dr. Morgan, in charge of the Electrical Department at the Royal Southern Hospital, who kindly advised us in the administration of the rays. The exposures to the rays were given by Miss Wells, an experienced worker, under the supervision of Dr. Morgan. The rat lived fifteen days, and we can therefore say that this animal's life was prolonged, when compared with the average life of twenty-two untreated rats, which was 11·3 days.

In spite of five exposures, each of twenty minutes duration, during which period the whole body of the rat was exposed to the direct action of the rays, the trypanosomes remained lively and increased steadily in number in the peripheral circulation.

The rays were not, therefore, trypanocidal in the exposures given by us, but, curiously enough, the life of the animal was prolonged.

We append table:—

RAT 50.—Piebald, weight 77 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day ...	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm. ...	—	—	6x	32,256	227,840	305,376	287,776	350,000
X-rays for 20 minutes ...	—				X-rays	—	X-rays	—

Day	9	10	11	12	13	14	15
Number of Trypanosomes per c.mm. ...	252,320	350,000	350,000	410,000	366,000	430,000	323,84
X-rays for 20 minutes ...			X-rays		X-rays	—	X-rays

The animal always seemed to brighten up during the exposure to the rays. The trypanosomes, it will be noted, increased in numbers in the peripheral circulation after each exposure, and we would conclude that there was no destruction of the trypanosomes. This is exactly in line with the experiments made by one of us (R.R.) several years ago, who found that exposure of the trypanosomes *in vitro* to the influence of X-ray had no trypanocidal action.

V. LEUCOCYTIC EXTRACT

This experiment was carried out at the suggestion of Dr Moore Alexander, Pathologist to the Royal Southern Hospital, and he very kindly prepared the extract for us.

A white rat, weighing 120 grams, was inoculated with the Rhodesian strain of trypanosomes, and the disease was allowed to incubate. On the twelfth day, when the trypanosomes numbered 89,000 per c.mm., we injected subcutaneously 0·5 c.c. of leucocytic extract. The following day the trypanosomes numbered 220,800 per c.mm., and the leucocytes rose from 8,160 per c.mm. to 10,764 per c.mm. The animal lived fourteen days. We cannot, therefore, draw conclusions here, and much further work will have to be undertaken before we conclude as to the value of leucocytic extract in trypanosomiasis.

We append table:—

RAT 51.—White, weight 120 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*.

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c mm.					48	27,720	76,960
Leucocytes				5,680			19,040
Leucocytic extract							

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	11,760	11,960	66.360	13.272	89,000	220,800	472,000
Leucocytes		40.296		–	8,160	10,764	—
Leucocytic extract			—	—	0·5 c.c.		

AUTO-AGGLUTINATION OF RED BLOOD CELLS IN TRYPANOSOMIASIS*

BY

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INTRODUCTION

It is now a well-recognised fact that the erythrocytes in fresh preparations of the blood of sleeping sickness cases and animals infected with trypanosomiasis frequently exhibit a more or less marked degree of agglutination. Attention was first drawn to this phenomenon in 1898 by Kanthack, Durham and Blandford,† who found that the red blood cells of animals infected with nagana, instead of forming rouleaux, tended to clump together into masses and to lose their outlines.

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* Reprinted from Proc. Roy. Soc., B. Vol. LXXXIII, p. 238.

† 'On Nagana or Tsetse Fly Disease,' Roy. Soc. Proc., 1898, Vol. LXIV, p. 100.

More recently Christy[*] (1904), Dutton and Todd[†] (1905), Martin, Lebœuf and Roubaud[‡] (1906-8), and others have described a similar condition in fresh preparations of the blood of patients suffering from sleeping sickness.

Dutton and Todd,[§] referring to this condition of the blood, wrote: ‘Only once have we had the opportunity of observing a ‘patient European’ from whose blood trypanosomes, once present, ‘have finally disappeared.’ In this instance auto-agglutination of the red cells disappeared with the parasites.

Later, it has been noted by many investigators[||] that the phenomenon gradually disappears in animals after the successful drug treatment of the disease.

In spite of the fact that auto-agglutination of the red cells in trypanosomal infections has attracted so much attention, very little work appears to have been done with a view to determining the nature of the changes in the blood which lead to its manifestation. Most of the workers have contented themselves with simply recording the presence of the phenomenon. So far as I have been able to ascertain from a search of the literature, only two authors mention any details regarding the mechanism of its production.

Kanthack, Durham and Blandford stated that the serum of the blood of animals exhibiting auto-agglutination, when added to normal blood of the same species of animal, caused the red corpuscles to clump together. On the other hand, at a recent meeting of the Society of Tropical Medicine and Hygiene, in the discussion following Dr. Bagshawe's paper on 'Recent Advances in our Knowledge of Sleeping Sickness,' Breinl[*] remarked that with regard to its (auto-agglutination) mechanism he had not, up to the present, been able to arrive at any definite conclusions. All his attempts to isolate a haemagglutinin had failed, and he had not been able to demonstrate either an iso- or an auto-agglutinin. Dr. Nierenstein[†] had shown that in trypanosomiasis a definite increase of the acidity of the blood occurred. This was most probably due to an increase of the amino-acids in the blood, and Breinl and Nierenstein inclined to the view that auto-agglutination was caused by this excess of amino acids. Unfortunately, they had not so far been able to find a definite proof of the correctness of this conception.

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[*] ‘Sleeping Sickness,’ British Medical Journal, 1904, p. 1456.

[†] ‘Gland Puncture in Trypanosomiasis,’ Memoir of the Liverpool School of Tropical Medicine, 1905, No. 16, p. 99.

[‡] Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Français, 1906-8, p. 281.

[§] *Loc. cit.*

[||] Thomas and Breinl, ‘Pathology and Treatment of Trypanosomiasis,’ Memoir of the Liverpool School of Tropical Medicine, 1905, No. 16.

Mesnil, Nicolle, and Aubert, ‘Recherches sur le Traitement des Infections Experimentales à *T. gambiense*,’ Annales de l'Institut Pasteur, 1907, Vol. XXI, p. 1.

[¶] Laveran and Thiroux, ‘Sur le Traitement des Trypanosomiases,’ Bulletin Soc. Path. Exot., 1908, p. 28.

Martin and Darré, ‘Sur les Symptomes Nerveux du Début de la Maladie du Sommeil.’ Bulletin Soc. Path. Exot., 1908, p. 15.

The results obtained by these investigators are thus somewhat conflicting, for whereas Kanthack, Durham, and Blandford found an iso-agglutinin in the serum of certain animals infected with *T. brucei*, Breinl was unable to demonstrate the existence of either an auto- or an iso-agglutinin.

EXAMINATION OF SERUM OF INFECTED ANIMALS FOR AUTO-AGGLUTININ

Having under observation a number of animals, the blood of which when examined in cover-slip preparations exhibited the phenomenon of auto-agglutination to a greater or less degree, it was decided to perform experiments with a view to investigating the mechanism of its production.

Technique.—The blood was collected in a solution containing 1 per cent. sodium citrate and 0·9 per cent. sodium chloride in distilled water. The blood was then centrifugalised and the corpuscles washed three times in normal saline solution. A 5 per cent. suspension of the washed red cells was then made in normal saline. Another sample of the same blood was collected and allowed to clot and the serum subsequently freed from the clot by centrifugalisation.

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[*] Trans. Soc. Trop. Med. and Hygiene, 1909, p. 29.

[†] 'Observations on the Acidity and Alkalinity of the Blood in Trypanosome Infections,' Annals of Tropical Medicine and Parasitology, 1908, p. 227

Equal amounts of the serum and emulsion of red corpuscles were then mixed together and drawn up into a capillary tube which was placed vertically in the incubator at 37° C.

The results of such experiments may be briefly summarised. With the exception of a few cases in which there appeared to be a trace of agglutination they were all negative. Similar negative results were obtained when the sera were examined for the presence of iso-agglutinins and also when the serum was replaced by citrated plasma.

At first one was somewhat at a loss to account for these apparently conflicting results, as even the sera of animals where the cover-slip preparations exhibited the most marked auto-agglutination of the red cells gave invariably negative results when examined for auto- and iso-agglutinins by this method.

Later it was observed that when the blood from one of the infected animals was allowed to flow from a vein of the ear into a watch glass containing a small amount of citrated saline solution, the red cells quickly sank to the bottom in little clumps, producing in a marked degree the sandy appearance described by Dutton and Todd.* When, however, the watch-glass and salt solution were warmed to 37° C. and the blood dropped in as previously, this appearance did not result; the red cells remained suspended for a considerable time and only subsided gradually, as in the case of normal blood. So long as the temperature of the watch-glass and salt solution was kept at 37° C., no agglutination resulted, but as soon as the temperature was allowed to fall to about 18° C., the red cells ran together into clumps and the typical sandy appearance was obtained.

These observations served to indicate that temperature played an important *rôle* in the development of the phenomenon. Accordingly, the previous experiment was repeated, but on this occasion three sets of tests were made: the first were placed in the incubator at 37° C., the second were left at laboratory temperature (18° to 21° C.), the last were kept in the ice chest at 0° C. Even at the end of five minutes a certain degree of agglutination of the red cells was noticeable in some of the tubes which had been placed in the ice chest, whilst in fifteen minutes the red cells in most of them were completely agglutinated, numerous clumps of various sizes being visible in the clear serum. The reaction was also distinct in many of the tubes kept at the laboratory temperature, but it was neither so marked, nor did it occur so quickly as in those subjected to the lower temperature. As before, no agglutination—or only occasionally a trace was observable in the tests which had been placed in the incubator at 37° C.

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* *Loc. cit.*

A large number of similar experiments were subsequently performed with the blood of monkeys, donkeys, goats, dogs, rabbits, guinea-pigs, and rats infected with various strains of trypanosomes. As a rule, it was found that a marked degree of agglutination only resulted when the temperature of the mixture of serum and red cells was lowered. Very exceptionally slight traces of agglutination were also seen in the tests carried out at 37° C., but these could not be compared with the intensity of the reaction at low temperatures.

Quite frequently a well-marked auto-agglutination was found to occur, at 0 C., in the control tests made with the blood of normal animals. I shall return to this important point later.

Iso-agglutination. A series of experiments were undertaken with a view to ascertaining whether the sera of those infected animals which possessed the property of agglutinating their own red cells were also capable of producing agglutination of the erythrocytes of other animals of the same species. In every case where auto-agglutination was present, iso-agglutination was found to occur when the serum of the infected animal was added to the red cells of another animal (either normal or infected) of the same species.

Analogous results were obtained with the blood of a case of human trypanosomiasis in Major Ross's clinic in Liverpool. Cover-slip preparations of the blood of this case exhibited a certain degree of auto-agglutination during the six months he was under observation. The following table gives the results of an examination of his blood, drawn ten days before his death, for auto- and iso-agglutinins.

TABLE I.—Examination of Sleeping Sickness Serum for Auto- and Iso-agglutinin

Equal volumes of serum and red blood cell suspension used.			
5 per cent. suspension of washed erythrocytes in normal saline solution	Source from which serum was obtained	Temp.	Result
		° C.	
Sleeping Sickness	Sleeping Sickness	37	No agglutination in 30 mins.
		18	Marked 30 ,,
		0	Complete .. 10 ,,
Normal individual A ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Complete ,, 30 ,,
		0	 10
Normal individual B ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Marked .. 30
		0	Complete . 10 ,,
Normal individual C ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Marked 30 .
		0	Complete .. 1 .
Normal individual C ...	Normal individual C	37	No agglutination in 30 ,,
		18	.. .. 30 ,,
		0	,, ,, 30 ,,
Normal individual C ...	Normal individual B	37	No agglutination in 30 ,,
		18	.. .. 30 ,,
			Marked .. 30 ,,

SPONTANEOUS AGGLUTINATION OF THE RED CELL SUSPENSIONS

On rare occasions it was found that the 5 per cent. suspension of red cells which had been washed three times in large volumes of 0·9 per cent. sodium chloride solution underwent a spontaneous agglutination in the entire absence of serum. Indeed, in one or two instances, where the animals exhibited an extreme degree of auto-agglutination, some difficulty was experienced in obtaining an even suspension of the erythrocytes. The probable explanation of this spontaneous agglutination is that it was due to the absorption of agglutinin from the plasma by the red cells immediately after the blood was shed into the cold citrated saline solution.

This difficulty was obviated by collecting the blood in warm citrate solution, and then rapidly centrifugalising and decanting off the citrated plasma. The red cells were then washed thrice in warm normal saline solution. Suspensions prepared in this way exhibited no tendency to spontaneous clumping.

It might be mentioned in this connection that Klein* has succeeded in obtaining agglutinating solutions by grinding up with quartz sand the well-washed erythrocytes of certain animals (rabbit, dog, hen, and guinea-pig). These extracts sometimes agglutinated the red cells of other animals, and frequently also the erythrocytes of the same kind of animal, and even those of the same animal.

ABSORPTION OF AGGLUTININ BY RED CELLS

Experiment.—To one volume of the citrated plasma of Rabbit 896 (infected with *T. dimorphon*), which caused great agglutination when added to its own red cells and to those of normal rabbits, were added five volumes of the undiluted well-washed red cells of the same animal. The mixture was then divided into two equal portions, A and B. A was placed in the incubator at 37° C. and B in the ice chest at 0° C. At the end of three hours the extracted plasmas were separated from the red cells by centrifugalisation, and were examined for auto- and iso-agglutinins.

TABLE II.

Equal volumes of extracted plasma and erythrocyte suspension used. Temp. of experiment 0° C.		Result
5 per cent. suspension of washed erythrocytes in normal saline solution.	Extracted plasma	
Rabbit 896	A	Complete agglutination in 10 mins.
,, ...	B	No agglutination in 60 mins.
Normal rabbit	A	Complete agglutination in 10 mins.
,, ...	B	Slight agglutination in 60 mins.

The plasma which had been in contact with the red cells at 0° C. had almost completely lost its agglutinating action, whilst the other portion, B, which had been placed in the incubator, had retained its auto- and iso-agglutinins intact.

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* 'Beiträge zur Kenntniss der Agglutination rother Blutkörperchen.' Wien. Klin. Woch., 1902, No. 16, p. 413.

The following observation furnishes additional proof of the capacity of erythrocytes to absorb haemagglutinin in the cold. Specimens of the blood of a number of infected animals were collected in sterile tubes and placed immediately in the ice chest to clot. After six hours the sera were separated from the clots by centrifugalisation and were examined for auto- and iso-agglutinins. In several cases the results were negative, no auto- or iso-agglutination being observed. In other instances the red cells were found to be clumped to a greater or less degree. The amount of agglutinin present in these sera was then compared with that occurring in the citrated plasma of the same animals which had been separated from the red cells at 37° C. The following procedure was adopted: The serum and plasma were diluted with gradually increasing amounts of 0·9 per cent. sodium chloride solution, and the degree of dilution observed at which they no longer caused complete agglutination of a given volume of the red cell suspension in a stated time.

The results obtained with one of the animals (Rabbit 1035), infected with *T. brucei*, are given in tabular form. It is to be observed that there was at least five or six times as much agglutinin in the plasma which had been separated from the red cells at 37° C. as in the serum obtained from the blood which had been allowed to clot in the ice box.

The sera of the other animals all showed a considerable deficiency in agglutinin as compared with that present in the plasma of the same animals.

When examining the blood for auto-agglutination it was found that the strongest reactions were obtained by dropping the blood into a very small quantity of warm citrated saline solution, and then separating the plasma from the red cells as speedily as possible with the centrifuge. A perhaps even more satisfactory method, and one in which the dilution of the plasma by the citrate is avoided, is to use the defibrinated plasma obtained by shaking the blood at a sustained temperature of between 37° and 40° C. in a bottle containing a few glass beads.

Table III.—Diminution of Amount of Auto- and Iso-agglutinin in Serum obtained from Blood which was allowed to Clot at [illegible]

Equal volumes of red cell suspension and diluted serum or plasma used. Temp. of experiment [illegible]

5 per cent. suspension of washed red cells of Rabbit 1035 (infected with *T. brucei*) and of normal rabbit	Serum of Rabbit 1035 obtained by allowing the blood to clot at [illegible] C. Plasma of Rabbit 1035 separated from the red cells at 37° C.	Result		
Rabbit 1035	Undiluted serum	Complete auto-agglutination in	15 min.	
	„ plasma	„ „	15	
Normal rabbit	„ serum	Complete iso-agglutination	15	
	„ plasma	„ „	15	
Rabbit 1035	Serum diluted with an equal volume of 0·9 per cent. NaCl solution	Complete auto-agglutination in	15	
	Plasma „ „ „	„ „	15	
Normal rabbit	Serum „ „ „	Complete iso-agglutination	15	
	Plasma „ „ „	„ „	15	
Rabbit 1035	Serum diluted with 2 volumes of 0·9 per cent. NaCl solution	Complete auto-agglutination in	15	*
	Plasma „ „ „	„ „	15	
Normal rabbit	Serum „ „ „	Complete iso-agglutination	15	
	Plasma „ „ „	„ „	15	
Rabbit 1035	Serum diluted with 3 volumes of 0·9 per cent. NaCl solution	Partial auto-agglutination in	30	
	Plasma „ „ „	Complete	30	
Normal rabbit	Serum „ „ „	Complete iso-agglutination	20	†
	Plasma „ „ „	„ „	20	
Rabbit 1035	Serum diluted with 4 volumes of 0·9 per cent. NaCl solution	Trace auto-agglutination in	30	
	Plasma „ „ „	Complete	30	
Normal rabbit	Serum „ „ „	Very marked iso-agglutination	30	
	Plasma „ „ „	Complete „	30	
Rabbit 1035	Serum diluted with 6 volumes of 0·9 per cent. NaCl solution	No agglutination in	30	
	Plasma „ „ „	Complete auto-agglutination	30	
Normal rabbit	Serum „ „ „	Slight iso-agglutination	30	
	Plasma „ „ „	Complete „	20	
Rabbit 1035	Serum diluted with 8 volumes of 0·9 per cent. NaCl solution	No agglutination in	30	
	Plasma „ „ „	Complete auto-agglutination	30	
Normal rabbit	Serum „ „ „	No iso-agglutination	30	
	Plasma „ „ „	Complete iso-agglutination	20	
Rabbit 1035	Plasma diluted with 12 volumes of 0·9 per cent. NaCl solution	Complete auto-agglutination in	30	‡
Normal rabbit	„ „ „ „	„ iso-agglutination	30	
Rabbit 1035	Plasma diluted with 14 volumes of 0·9 per cent. NaCl solution	Partial auto-agglutination in	30	
Normal rabbit	„ „ „ „	Complete iso-agglutination	30	§
Rabbit 1035	Plasma diluted with 16 volumes of 0·9 per cent. NaCl solution	No agglutination in	30	
Normal rabbit	„ „ „ „	Slight iso-agglutination	30	

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* Denotes the end point of the auto-reaction of serum.
‡ „ „ „ „ plasma.

† Denotes the end point of iso-reaction of serum
§ „ „ „ „ plasma.

Well marked agglutination was frequently observed when the plasma obtained in this manner was added to a 5 per cent suspension of red cells, whilst either a negative or only slightly positive result was obtained with the serum derived from blood which had clotted at laboratory temperature or in the ice chest.

REVERSIBILITY OF THE REACTION

In view of the fact that red cells absorb agglutinin to a much greater extent at low temperatures and only slightly at higher temperatures (37° to 40° C.), the question arises as to whether the absorption of agglutinin belongs to the group of reactions which have been designated 'reversible.' In other words, will raising the temperature of the agglutinated masses of red cells cause the clumps to disintegrate into their corpuscular elements?

Experiment.—One volume of red corpuscles of Rabbit 1022 (infected with *T. gambiense*) was added to twenty volumes of the defibrinated plasma of the same animal. After an hour's sojourn in the ice chest complete agglutination of the erythrocytes was found to have occurred. After stirring the clumps up thoroughly with a glass rod a small drop of the suspension was placed on a cover-slip and a hanging drop preparation made. On examining with the microscope large masses of agglomerated red blood cells and also considerable rouleaux formation were seen. The mixture of clumped red cells and defibrinated plasma was now placed in the incubator at 37° C. In about fifteen minutes the clumps were no longer visible, and the erythrocytes appeared to be evenly suspended throughout the fluid. A hanging drop preparation was made on a warm slide and cover-slip. As long as the temperature was maintained at 37° C. there was no tendency to agglutination. When the suspension was again cooled to 0° C. agglutination of the red cells reappeared after a few minutes.

From this and similar experiments it follows that the reaction is reversible, the phenomenon disappearing on warming and reappearing on cooling.

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[*] Arrhenius, Immuno-Chemistry, ch. 2.

AUTO-AGGLUTINATION IN THE BLOOD OF NORMAL ANIMALS

Attention has already been drawn to the fact that a certain amount of auto-agglutination was frequently observed in the control tests of normal blood. Some years ago Klein[*] found auto-agglutinin to be present in the serum of a number of normal horses.

Landsteiner[†] demonstrated the existence of a similar substance in the blood of rabbits, horses, dogs, and cattle.

Other writers, on the contrary, deny the existence of auto-agglutinin in normal blood. Dudgeon[‡] in a recent paper states that auto-agglutination does not occur in normal human blood.

It was decided to re-investigate this subject more fully, using the blood of a considerable number of normal animals of different kinds.

Technique. The blood was obtained from a convenient vein, and the defibrinated plasma separated from the red corpuscles at 37° C. in the manner already described. The red corpuscles were washed three times in warm saline solution, and finally a 5 per cent. suspension made in 0·9 per cent. sodium chloride solution. Equal volumes of the defibrinated plasma and red cell suspension were drawn up together into three fine pipettes which were then subjected to a temperature of 0°, 15°, and 37° C. respectively. The pipettes were kept in the vertical position, and the contents examined for auto-agglutination with the aid of a lens from time to time. It was found in the majority of cases that the test could not well be continued for longer than one hour, owing to the fact that in most cases the erythrocytes had subsided to a marked degree after the lapse of this period. At times the citrated plasma was substituted for the defibrinated plasma. The same precautions regarding temperature were taken, and only very small amounts of citrate solution (not more than a tenth of the volume of plasma) employed. The plasma obtained in this way frequently clotted, but the process was sufficiently retarded to permit of the previous separation of the red corpuscles. No appreciable difference between the agglutinating action of the defibrinated and citrated plasma was observed.

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[*] 'Beitrage zur Kenntniss der Agglutination rother Blutkorperchen,' Wien. Klin. Woch., 1902, No. 16, p. 413.

[†] 'Ueber Beziehungen zwischen dem Blutserum und den Korperzellen,' Munch. Med. Woch., 1903, No. 42.

[‡] 'On the Presence of Haemagglutinins, etc., in the Blood obtained from Infectious and Non-Infectious Diseases in Man,' Roy. Soc. Proc., 1909, B, Vol. LXXXI, p. 207.

The results of this investigation of normal blood for auto-agglutinin may be summarised by stating that small quantities of auto-agglutinin were found to be present in the blood of rabbits (14), guinea-pigs (4), goats (3), dogs (2), horses (4), donkeys (2), monkeys (*Macacus rhesus*) (2), and *Cercopithecus callitrichus* (2). Sometimes, especially in goats and guinea-pigs, the amount present was exceedingly small, and considerable care was necessary to demonstrate its existence. In these cases a larger volume of serum was used in proportion to the amount of red cells and the reaction allowed to proceed for a longer period. It is to be observed that clumping of the erythrocytes only occurred in the tests carried out at low temperatures, and not in those subjected to a temperature of 37° C.

RELATION OF AUTO-AGGLUTININ OF NORMAL BLOOD TO THAT PRESENT IN THE BLOOD OF ANIMALS INFECTED WITH TRYPANOSOMES

In this connection it may be remarked that in the blood of infected animals there exists a considerable excess of auto-agglutinin beyond that present in the blood of normal animals. It was found that diluting the defibrinated plasma of normal blood with twice its volume of normal saline solution usually sufficed to destroy its agglutinating action. On the other hand, it was often possible to dilute the infected plasma 15- or 20-fold, and still obtain complete agglutination of the erythrocyte suspension.

EFFECT OF HEAT ON AUTO-AGGLUTININ

Different portions of the defibrinated plasma of normal and infected animals were heated in a water bath to 58° C. and 70° to 72° C. respectively for twenty minutes. Heating to 58° C. was found not to destroy auto-agglutinin, whereas plasma which had been subjected to a temperature of 70° C. for twenty minutes had completely lost this property.

SIGNIFICANCE OF THE PHENOMENON IN TRYPANOSOMAL INFECTIONS

The question of the mechanism of production of auto-agglutination in trypanosomal infections is one which has frequently been discussed, but as yet no satisfactory explanation has been offered. With reference to this question it appears to me that two theories might be advanced to explain the development of an excess of auto-agglutinin in this disease.

It has long been recognised that the blood of men and the lower animals suffering from trypanosomaisis is frequently very anaemic. Both the percentage of haemoglobin and the number of red corpuscles per cubic millimetre fall to a low level. This is particularly the case in the last stages of the disease. Conceivably auto-agglutinin might develop in the plasma as a result of auto-inoculation of an animal resulting from the destruction of its own erythrocytes.

There are, however, many considerations which operate against this view. In the first place I have found no constant relation between the development of anaemia and auto-agglutination of the red cells. By the aid of systematic haemocrit examinations of the blood of recently infected animals it was observed that auto-agglutination was usually pronounced for a considerable period before any marked fall of the haemocrit value had occurred. Secondly, a marked degree of auto-agglutination comparable to that occuring in trypanosomiasis has not been described in any other of the diseases in which anaemia is a distinctive feature. Dudgeon* examined the blood of twenty-six cases of anaemia due to various causes without finding a single example of auto-agglutination. It is doubtful, however, whether the technique adopted by Dudgeon is suitable for the recognition of small amounts of auto-agglutinin. Then, again, it is generally recognised as impossible to evoke the production of auto-bodies experimentally by inoculating an animal with its own tissues.

Experiment. A rabbit was injected intraperitoneally with 10 c.c. of its own erythrocytes which had been laked with distilled water and the resulting solution made isotonic with sodium chloride.

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* 'On the Presence of Haemagglutinins, etc., in the Blood obtained from Infectious and Non-infectious Diseases in Man,' Roy. Soc. Proc., 1909, B, Vol. LXXX, p. 531.

This injection was repeated after an interval of a week. No increase of auto-agglutinin was found to occur in the animal's blood.

Another possible explanation for the cause of the development of auto-agglutinin is that it is formed by the animal mechanism as a direct response to the stimulus of the pathogenic agent. In the consideration of this question it is necessary to inquire whether auto-agglutinin alone is present in excess in infected blood or whether we have at the same time a corresponding alteration in the iso- and hetero-agglutinin contents of the serum.

Mention has already been made of the fact that in every case where the serum of an infected animal was found to possess the property of clumping its own erythrocytes to a considerable degree, it also agglutinated markedly those of other members of the same species.

It was further observed that the plasma of infected animals frequently appeared to agglutinate the red cells of animals belonging to different species to a greater extent than normal. The case of human trypanosomiasis already referred to presented an excellent example of an increased capacity on the part of an infected serum to agglutinate foreign erythrocytes. The serum of this case clumped the red corpuscles of rats, guinea-pigs and rabbits in a remarkable manner. A few drops of the inactivated serum when added to an equal volume of the blood of one of these animals caused intense agglutination in a few seconds at room temperature. The action of normal human sera on these corpuscles was much slower and did not approach that of the former in intensity.

Experiments were undertaken with the object of comparing quantitatively the auto-, iso-, and hetero-agglutinin in the blood of several infected animals with that existing in the blood of normal animals of the same kind.

Technique.—The method adopted was that previously used for comparing the amount of auto- and iso-agglutinin in the defibrinated plasma separated from the red cells at 37° C. with that present in the serum obtained from blood which had clotted at 0° C.

Inactivated defibrinated plasma was prepared from normal

Rabbit 1 and from Rabbit 896 (infected with *T. dimorphon*), and a 5 per cent. suspension of washed erythrocytes from the following animals: normal Rabbits 1 and 2, Rabbit 896 and a normal horse and guinea-pig.

From the results of this experiment, details of which are given in Table IV, and from other observations of the same kind one is led to conclude that in the blood of infected animals, in addition to an excess of auto-agglutinin, there is also frequently a corresponding increase in iso- and hetero-agglutinin.

The question now arises as to whether these reactions are manifestations of the same body or of different specific agglutinins. The procedure usually adopted for the solution of problems of this nature is the saturation of a portion of the serum by the red cells of one of the varieties in question, and then, after allowing reaction to take place for some hours, centrifugalising and examining the extracted serum regarding its agglutinating action on the red cells of the kind used for extraction and also on the other varieties of erythrocytes.

Malkoff, adopting this technique, arrived at the conclusion that there exist in goat's serum, which is capable of agglutinating the erythrocytes of many kinds of animals, different specific agglutinins, each of which has a specific affinity for the corresponding variety of red cells.

Experiment. (1) One volume of defibrinated plasma of Rabbit 896 (infected with *T. dimorphon*) was extracted for twelve hours at 0° C. with an equal volume of the undiluted red blood cells of the same animal. (Extracted plasma A.) (2) Here the proportion of plasma to erythrocytes was one to five. (Extracted plasma B.) (3) One volume of the same plasma was treated with one volume of normal horse's red cells. (Extracted plasma C.)

A 5 per cent. suspension of washed erythrocytes was prepared from the following animals: Rabbit 896, normal Rabbit 1, normal Donkeys 1 and 2, a normal guinea-pig, horse, *Macacus rhesus*, *Cercopithecus callitrichus*, and human being.

The results indicate that complete extraction of the infected plasma of Rabbit 896 by its own erythrocytes and those of a normal

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[*] 'Beitrage zur Frage der Agglutination von rother Blutkorperchen.' Deutsche Med. Woch., 1900, No. 14.

TABLE IV.—Comparison of the Amount of Auto-, Iso-, and Hetero-agglutinin in the Plasma of Rabbit 896 (infected with *T. dimorphon*) and Normal Rabbit 1

Equal volumes of red cell suspension and diluted plasma used. Temperature of experiment 0° C.

5 per cent. suspension of washed erythrocytes in normal saline solution	Plasma of Rabbits 1 and 896 diluted with increasing amounts of normal saline solution	Result
Rabbit 1	Rabbit 1 undiluted	Complete agglutination in 20 mins.
„ 1	„ 896 „	„ „ 20 „
„ 2	„ 1 „	„ „ 20
„ 2	„ 896 „	„ „ 20 „
„ 896	„ 1 „	„ „ 20 „
„ 896	„ 896 „	„ „ 20 „
Horse	„ 1 „	„ „ 10
	„ 896 „	„ „ 10
Guinea-pig	„ 1 „	Marked „ 45 „
	„ 896 „	Complete „ 45
Rabbit 1	„ 1 diluted with twice its vol. of 0·9 per cent. NaCl solution	Nil „ 45 „
„ 1	„ 896 „	Complete „ 20
„ 2	„ 1 „	Slight „ 20
„ 2	„ 896 „	Complete „ 20 „
„ 896	„ 1 „	Nil „ 20 „
„ 896	„ 896 „	Complete „ 20 „
Horse	„ 1 „	Marked „ 45 „
	„ 896 „	Complete „ 15
Guinea-pig	„ 1 „	Nil „ 45 „
	„ 896 „	Complete „ 15 „
Rabbit 1	„ 1 diluted with 4 times its vol. of 0·9 per cent. NaCl solution	Nil „ 15 „
„ 1	„ 896 „	Complete „ 15 „
„ 2	„ 1 „	Nil „ 15 „
„ 2	„ 896 „	Complete „ 30 „
„ 896	„ 1 „	Nil „ 30 „
„ 896	„ 896 „	Complete „ 30 „
Horse	„ 1 „	Slight „ 30 „
	„ 896 „	Complete „ 30 „
Guinea-pig	„ 1 „	Nil „ 30 „
	„ 896 „	Complete „ 20 „
Rabbit 1	„ 896 diluted with 6 times its vol. of 0·9 per cent. NaCl solution	„ „ 20
„ 2	„ 896 „	„ „ 30 „
„ 896	„ 896 „	„ „ 30
Horse	„ 896 „	Marked „ 45 „
Guinea-pig	„ 896 „	Complete „ 15 „
Rabbit 1	„ 896 diluted with 9 times its vol. of 0·9 per cent. NaCl solution	„ 20 „
„ 2	„ 896 „	Partial „ 45 „
„ 896	„ 896 „	Complete „ 30
Horse	„ 896 „	Trace „ 45 „
Guinea-pig	„ 896 „	Complete „ 15
Rabbit 1	„ 896 diluted with 14 times its vol. of 0·9 per cent. NaCl solution	Slight „ 45 „
„ 2	„ 896 „	Nil „ 45 „
„ 896	„ 896 „	Slight „ 45 „
Horse	„ 896 „	Nil „ 45 „
Guinea-pig	„ 896 „	Slight „ 45 „

horse does not completely destroy the agglutinating action of the plasma on the red blood cells of other animals, although it is to be noted that in most cases when the plasma had been extracted with five times its volume of its own red cells there was a marked lessening or even total disappearance of this action. This diminution of the agglutinating action of the plasma cannot be explained by mere dilution with the small amount of saline solution adhering to the red cells, as the plasma still caused marked agglutination after the addition of fifteen times its volume of 0·9 per cent. sodium chloride solution.

It is doubtful, however, whether experiments of this kind really have the importance that has been assigned to them by Malkoff and others.

Landsteiner and Sturli* using normal horse and dog serum and eleven varieties of erythrocytes, confirmed Malkoff's observation that saturation of the serum with one kind of red blood cell deprived it of the power to agglutinate this variety, and this only. They furthermore showed that red cells which had already been once completely agglutinated were still able to react with another kind of serum, and that the new serum after the reaction had lost its power to agglutinate fresh corpuscles of the same kind. Hence, as Landsteiner points out, the problem had assumed a very complex aspect, the enormous number of specific agglutinins in normal serum appearing uneconomic.

Landsteiner and Sturli suggest another hypothesis to explain these facts, namely, that during the process of agglutination some substance passes from the red cell to the serum, and that after complete agglutination the serum, in consequence of the combination, agglutinin + corpuscle substance, can no longer react with red cells of the same kind, but can with those of other animals. By this theory they maintain that the facts can be explained without the necessity for assuming the presence of an enormous number of differently acting substances or groups of substances in normal serum.

A certain amount of support is afforded this view by the observation of Landsteiner that a watery extract of the corpuscles of a turkey, when added to horse serum, almost completely prevents its agglutinating action on the red cells of the turkey, but only in

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* 'Ueber die Hamagglutinine normaler Sera,' Wien. Klin. Woch., 1902, p. 38.

TABLE V.—Agglutinating Action of Plasma which had been previously Extracted with Red Blood Cells

Equal volumes of red cell suspension and plasma used.
Temp. of experiment 0° C.

5 per cent. suspension of washed erythrocytes in normal saline solution	Untreated plasma of Rabbit 896 (infected with *T. dimorphon*) and also plasma of the same animal extracted with red cells as follows: A, with an equal volume of red blood cells of Rabbit 896 B, with five times its volume of red cells of Rabbit 896. C, with an equal volume of red cells of a normal horse	Result	
Rabbit 896	Untreated plasma	Complete agglutination	in 10 mins
	Extracted .. A	Partial ..	45 ,,
	,, .. B	No ,,	45
	,, ,, C	Complete ..	15 ,,
Rabbit 1	Untreated plasma	Complete ..	15
	Extracted ,, A	,, ..	30 ,,
	,, .. B	No ..	45 ,,
	,, ,, C	Complete ,,	15 ,,
Donkey 1	Untreated plasma	Complete ..	15 ,,
	Extracted .. A	Almost complete ..	45 ,,
	,, .. B	No ,,	45 ,,
	,, .. C	Marked ..	45 ,,
Donkey 2	Untreated plasma	Complete ..	15
	Extracted ,, A	,, ..	30 ,,
	,, ,, B	Trace ..	45 ,,
	,, ,, C	Complete ..	45 ,,
Guinea-pig	Untreated plasma	Complete ..	15 ,,
	Extracted ,, A	,, ..	15 ,,
	,, .. B	Partial ..	45 ,,
	,, .. C	Complete ..	15 ,,
Horse	Untreated plasma	Complete ..	10 ,,
	Extracted ,, A	,, .	15
	,, ,, B	Slight ..	45 ,,
	,, ,, C	Partial ..	45
Macacus rhesus	Untreated plasma	Complete ..	15 ,,
	Extracted ,, A	,, ..	30 ,,
	,, ,, B	Slight ..	45 ,,
	,, .. C	Complete ..	30 ,,
Cercopithecus callitrichus	Untreated plasma	Complete ..	15 ,,
	Extracted ,, A	,, ..	15 ,,
	,, .. B	,, ..	30 ,,
	,, .. C	,, ..	15 ,,
Human being	Untreated plasma	Complete ..	10 ,,
	Extracted ,, A	,, ..	20 ,,
	,, ,, B	Marked ..	45 ,,
	,, .. C	Complete ..	15 ,,

a very slight degree on other kinds of blood. This last observation was subsequently confirmed by Lazar.* However, unless all traces of the stromata of the red cells had been removed from the haemoglobin solution—and this is by no means an easy performance—an obvious explanation for this inhibiting action of such solutions would be that the stromata themselves had fixed the agglutinins present in the horse serum, and consequently there would be little, if any, left to act upon the red corpuscles. Naturally, in this case, the inhibiting action would be specific for the variety of red cells from which the haemoglobin solution was made.

The fact that the phenomenon of auto-agglutination is reversible allows one to approach the subject of specificity from a different point of view, namely, by extracting the completely agglutinated red cells with a small quantity of normal saline solution at 37° C., and then investigating the nature of the digest.

Experiment.—To 10 c.c. of defibrinated plasma of Rabbit 1035 (infected with *T. brucei*) were added 0·2 c.c. of the red cells of the same animal. After allowing the mixture to stand in the ice chest for twelve hours with occasional stirrings the supernatant plasma was decanted off, and the clumped red blood cells washed four times with at least ten times their volume of normal saline solution at 0° C.; 0·2 c.c. of normal salt solution was then added to the agglutinated mass of red cells and the mixture allowed to digest at 40° C. for half an hour. At the end of this time no trace of agglutination was visible. The red cells were then quickly thrown down by centrifugalisation and the supernatant fluid removed (Digest solution).

A 5 per cent. suspension of red blood cells in normal saline solution was prepared from the following animals: Rabbit 1035 (infected with *T. brucei*), normal Rabbit A, Donkey 2 (infected with *T. rhodesiense*), normal Donkey A, Goat 1041 (infected with *T. rhodesiense*), normal Goat A, and from a normal rat, guinea-pig, dog, horse, *Macacus rhesus*, *Cercopithecus callitrichus*, and human being.

The capacity of the untreated plasma of Rabbit 1035 and of the solution prepared by digesting the agglutinated red cells with normal saline at 0° C. to agglutinate these different erythrocytes was then examined.

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* 'Ueber die Bedeutung der lipoiden Stoffe der rothen Blutkorperchen für den Mechanismus der Agglutination,' Wien. Klin. Woch., 1905, p. 1012.

TABLE VI.—Agglutinating Action of a Solution obtained by Digesting Auto-agglutinated Red Blood Cells with Normal Saline Solution at 40° C.

Equal volumes of red cell suspension and defibrinated plasma or digest solution used. Temperature of experiment 0° C.		
5 per cent. suspension of washed erythrocytes in normal saline solution	Untreated defibrinated plasma of Rabbit 1035 (infected with nagana), and solution obtained by digesting the auto-agglutinated red cells of the same animal with normal saline solution at 37° C.	Result
Rabbit 1035 (infected with *T. brucei*)	Defibrinated plasma	Complete agglutination in 10 mins.
	Digest solution ...	" " 10 "
Normal Rabbit A	Defibrinated plasma	" " 10 "
	Digest solution ...	" " 10 "
Donkey 2 (infected with *T. rhodesiense*)	Defibrinated plasma	" " 10 "
	Digest solution ...	" " 30 "
Normal Donkey A ..	Defibrinated plasma ..	" " 15 "
	Digest solution	Slight " 45 "
Goat 1041 (infected with *T. rhodesiense*)	Defibrinated plasma ...	Complete " 10 "
	Digest solution ...	No " 45 "
Normal Goat A	Defibrinated plasma	Complete " 10 "
	Digest solution	No " 45 "
Rat ...	Defibrinated plasma ..	Complete " 15 "
	Digest solution ..	" " 20 "
Guinea-pig ..	Defibrinated plasma ..	" " 15 "
	Digest solution	" " 20 "
Dog	Defibrinated plasma ..	" " 5 "
	Digest solution ...	" " 10 "
Horse	Defibrinated plasma ...	" " 10 "
	Digest solution ...	Partial 45
Macacus rhesus ..	Defibrinated plasma	Complete 15
	Digest solution	" " 30
Cercopithecus callitrichus ...	Defibrinated plasma	" " 15
	Digest solution ...	" " 15 "
Human being	Defibrinated plasma	" " 10 "
	Digest solution	" " 15 "

N.B.—The four specimens of normal saline solution which had been used for washing the clumped red cells were also examined. With the exception of occasional traces in the first, no agglutinin was found in these solutions.

The information obtained from observations of this kind is extremely interesting. In the experiment recorded a substance was extracted from the auto-agglutinated erythrocytes of a rabbit infected with *T. brucei* which clumped not only its own erythrocytes and those of other rabbits, but also the red cells of many other animals of different species. In other words it would appear that the auto-agglutinin is not a body equipped with a high degree of specificity, but that it can also act as iso- and hetero-agglutinin on the erythrocytes of other rabbits and those of animals of widely different species.

VALUE OF THE PHENOMENON AS A DIAGNOSTIC SIGN

Before discussing this question it is necessary to emphasise the importance of careful observation in determining whether a certain blood really agglutinates or not. So far as can be gathered from the papers in which the existence of the phenomenon in trypanosomiasis has been recorded, it has been invariably decided from the examination of cover-slip preparations of the blood. Although a considerable degree of auto-agglutination is easily recognised in a well-made cover-slip preparation, yet it is often extremely difficult, or even impossible, to decide whether the red cells are really agglutinated when the phenomenon is not so distinct. A certain amount of massing together of the erythrocytes is frequently evident at the edges of even the best cover-slip preparations of normal blood, whereas if the slide and cover-slip be not perfectly clean the red cells are found to be anything but evenly distributed, but are grouped together into little masses and rouleaux, separated from one another by plasma—an appearance closely resembling that to be observed in infected blood when the amount of auto-agglutination is slight. On the other hand, a slight degree of auto-agglutination can be easily obscured by pressure on the cover-slip resulting in the separation of the erythrocytes one from the other.

Furthermore, it has been shown that small amounts of auto-agglutinin exist constantly in the blood of many normal animals. In horses and donkeys auto-agglutinin is sometimes present in such an extent as to give rise to a more or less characteristic appearance in cover-slip preparations. This is specially the case when the preparations are made out of doors at a somewhat low temperature. However, I have never observed in cover-slip preparations of the blood of normal animals a condition approaching in intensity the well-marked clumping obtaining in infected cases. When a high degree of auto-agglutination exists the corpuscles are seen to have become agglomerated into tight clumps, the outlines of the individual cells being indistinct, or even completely lost, so that the clumps appear to consist of red cells which have fused together into a homogeneous mass. In order to evoke as characteristic an appearance as possible the preparation should be made at the lowest temperature practicable.

The next point to be considered is whether auto-agglutination is a constant feature in trypanosomal infections.

Martin, Lebœuf, and Roubaud[*] stated that in the large number of cases of human trypanosomiasis examined by them in the French Congo, auto-agglutination was always present. In the tables appearing in their report, the condition of the blood as regards auto-agglutination is indicated by numbers from 0 to 10, the cipher meaning that there is no agglutination, whereas the greatest degree of agglutination is indicated as 10; the intermediate figures denote intermediate degrees of agglutination.

In view of the technique used by them the mere examinations of cover-slip preparations of the fresh blood—such a classification appears to be a somewhat unwarrantable refinement.

Todd[†] in a recent paper classifies as regards auto-agglutination a large number (1406) of cases examined by Dutton and himself in the Congo Free State. Of the 395 cases in which auto-agglutination was present, trypanosomes were found in only 183. However, as Todd himself states, probably because of the insufficient search for them (the cases were seen and examined on one occasion only), trypanosomes were present much more often than they were found.

Later in the same paper it is stated that only in three cases were trypanosomes not present when an extremely well-marked auto-agglutination was recorded. One of these was a case of relapsing fever; another was a much emaciated marasmic individual, and the third was a case of syphilis.

Regarding the frequency of the phenomenon in the blood of experimentally infected animals, it need only be stated that as a rule auto-agglutination is best marked in the blood of the larger animals, *e.g.*, horse and donkey. It is usually also very distinct in the monkey, dog, rabbit, and goat. In the rat, mouse, and guinea-pig it is generally slight or absent.

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[*] Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Française, 1906-8 p. 281.

[†] 'A Note on the Occurrence of Auto-agglutination of the Red Cells in Human Trypanosomiasis,' Bull. Soc. Path. Exot., 1910, p. 438.

In the ordinary course of events the infected animal shows parasites in its blood for some time before a distinct auto-agglutination develops. Occasionally, however, auto-agglutination appears before trypanosomes have been found in the blood. I have had under observation a goat, infected with *T. gambiense*, in which trypanosomes have never been seen in the blood, but in which a well-marked auto-agglutination had developed. The blood was shown to be infective by injection into rats.

The last point to be decided in considering the value of the phenomenon as a diagnostic sign is whether it occurs in other diseases besides trypanosomiasis. In addition to the three cases mentioned by Todd, auto-agglutination has occasionally been noted in persons suffering from other diseases than sleeping sickness.

Klein,[*] in 1890, found auto-agglutination of the red blood cells in a case of hepatic cirrhosis (Hanot). Dudgeon[†] mentions the case of a West Indian negro which was considered to be one of tertiary hepatic syphilis, where the blood exhibited spontaneous clumping. He also found auto-agglutinin in the blood of a case of long standing epilepsy. Martin and Darré[‡] assert that the phenomenon is to be met with in certain forms of icterus due to haemolysis.

Quite recently Nattan-Larrier[§] described the existence of auto-agglutination in rats infected with the *Spirilla obermeiei*. It is interesting to note in this connection that of Todd's three cases which exhibited well marked auto-agglutination, but were not infected with trypanosomes, one was a case of relapsing fever and another a case of syphilis, as was also the only definite instance of the phenomenon seen by Dudgeon. It is of importance to know whether auto-agglutination is often present in spirochaetal infections.

In conclusion it may be stated that in the light of the information obtainable a well-marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence, apart from infection with trypanosomes.

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[*] 'Ueber die Untersuchung der Formelemente des Blutes und ihre Bedeutung fur die praktische Medicin,' Wien. Klin. Woch., 1890, Nos. 36-40.

[†] *Loc. cit.*

[‡] Bull. et Memoires Soc. Med. des Hôpitaux de Paris, 1909, p. 599.

[§] 'L'Autoagglutination des Hématies dans la Spirillose Expérimentale,' Bull. Soc. Path. Exot. 1910, p. 425.

SUMMARY

Auto- and iso-agglutinin are present in the blood of cases of sleeping sickness and of animals infected with trypanosomiasis

Reaction between auto-agglutinin and red blood cells takes place only at low temperatures.

Auto-agglutinin can be removed from plasma by absorption with the erythrocytes of the same animal at 0° C.

The reaction between auto-agglutinin and red blood cells is reversible.

Auto-agglutinin exists in small amounts in the blood of many normal animals.

Auto-, iso- and hetero-agglutinin are frequently present in much greater amount in the blood of infected animals than in that of normal animals, and it is due to this fact that clumping of the red blood cells is often visible in fresh cover-slip preparations of the blood of infected animals.

From the red blood cells of an infected animal which have been agglutinated in the cold by the plasma of the same animal an active substance can be extracted with normal saline solution at 37° C.

This substance agglutinates not only the red cells of the same animal and other members of the same species, but also those of many animals of different species.

It is to be inferred from the information at present available that a marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence apart from an infection with trypanosomes.

ON A NEW GENUS OF CULICINAE FROM THE AMAZON REGION

BY
R. NEWSTEAD, M.Sc., &c.,
AND
H. F. CARTER.

(*Received for publication* 15 *February*, 1911)

Thomasina, nov. gen. (Newstead and Carter)

Palpi of the male (fig. 1) much shorter than the proboscis; they are also rather slender and composed of four segments, of which the terminal one is very short and narrower than the preceding one. *Antennae* (fig. 1) rather sparsely clothed with

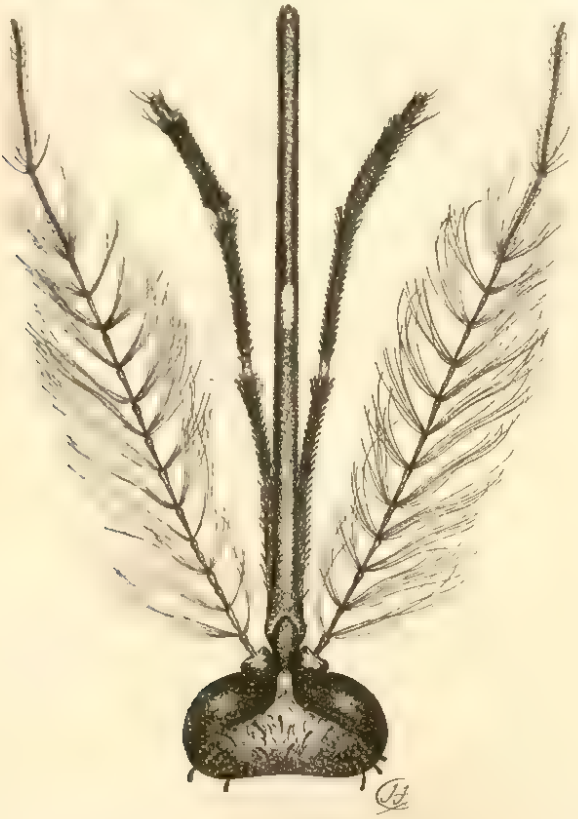

Fig. 1. Head of ♂ *Thomasina longipalpis*. (Newstead and Thomas.)

hairs. *Legs* with long and somewhat outstanding scales at the apex of the femora; the fourth and fifth segments of the front and middle tarsi very short and broad, being compressed laterally.

Scale structure of both sexes. Those of the head and thorax as in *Mansonia*, with the exception of a few broad curved ones just in front of the scutellum and at the base of the wings; pleurae densely clothed with large, broad, spindle-shaped scales, prothoracic lobes distally clothed with spindle-shaped ones, scutellum with long falciform scales. *Wings* with *Mansonia*-like scales, the outstanding ones being, however, much longer.

Type, *Thomasina longipalpis* (Newstead and Thomas).

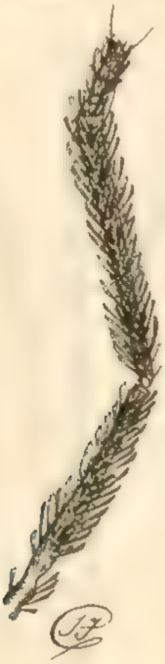

Fig. 2. Palpus of ♀ *Thomasina longipalpis*. (Newstead and Thomas.

In describing the female of this species, Newstead and Thomas* referred it to the genus *Mansonia* owing to a general resemblance which the scale structure bears to this genus; and, although these authors had noted the peculiar character of the palpi, they did not think this was of sufficient importance to warrant the erection of a new genus for the reception of this insect.

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* Ann. of Trop. Med. and Parasit., Vol. IV, No. 1. p. 145.

Having recently received several examples of both sexes, it was at once seen that the morphological characters of the palpi and tarsi were so markedly different from those of *Mansonia* that the species could no longer remain in that genus.

The marked characteristics of *Thomasina* are that the *palpi of the male are short, while those of the female are relatively long* (figs. 1 and 2), the latter almost one-third the length of the proboscis; and that *the fourth tarsal segment of the fore and mid-legs, in both sexes, is very small and flattened* (fig. 3, *a*, *b*).

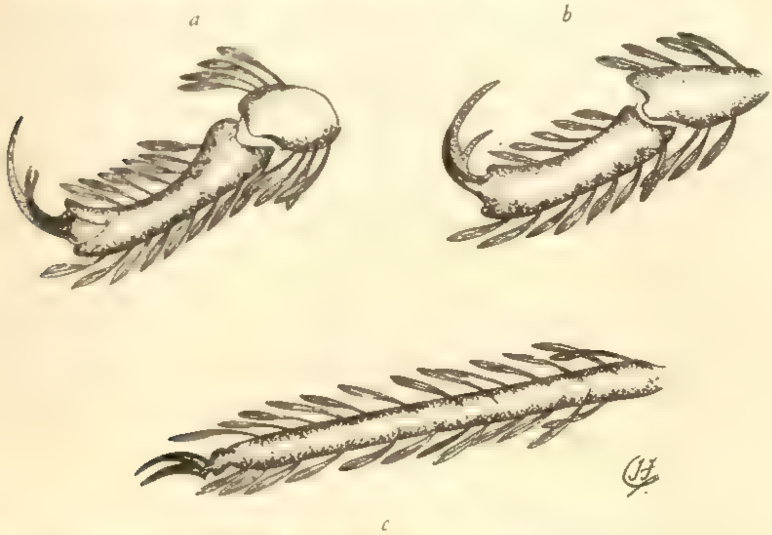

FIG. 3. *a* and *b*. Fourth and fifth tarsal segments and ungues of the fore and mid legs of ♂ *Thomasina longipalpis*. (Newstead and Thomas.)

c. Fifth segment and ungues of the same.

We have much pleasure in dedicating this insect to our colleague, Dr. H. Wolferstan Thomas, who has contributed so much towards our knowledge of the mosquitos of the Amazon region at Manaos and elsewhere.

Thomasina longipalpis (Newstead and Thomas) (1910).
Mansonia longipalpis (♀ only) (Newstead and Thomas) (1910)*.

MALE.—Colour and general characteristics as in the female. *Palpi* clothed with black, white, and ochreous-yellow scales, the

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* Ann. of Trop. Med. and Parasit., Vol. IV, No. 1, p. 145.

latter predominating; the articulations, the apical segment, and base of the first segment, white scaled. *Proboscis* very thick, slightly stouter than that of the female, but as far as can be seen, with the same distribution of scales. *Legs* (fig. 3), fourth tarsal segments of the first pair of legs slightly broader than long, in the ♀ larger and considerably longer than broad; ungues unequal, the larger with a distinct sub-median tooth, the smaller simple. Fourth segments of the second pair of legs longer and narrower than in the first pair, in the ♀ shorter and slightly broader and about the same size as the corresponding ones of the ♂; ungues as in the fore legs. Tarsi of the hind legs normal in both sexes, the ungues equal and simple. *Genitalia*:—Basal segment stout, gradually tapering to a rounded apex; superior clasper slender, about half the length of the basal lobe, with a long terminal spine; inferior clasper forming a fairly distinct lobe bearing a pair of thick spines. Harpes and harpagones stout, the former terminating in three distinct teeth, the latter in a single large one.

In addition to the female characters given by Newstead and Thomas, and besides those mentioned above, there are two others of some importance, viz., the ungues are all equal and simple, and in several specimens the ventral surface of the last tarsal segment of the hind legs is black scaled.

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Altogether twenty one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

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By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fourth number of the fourth volume.

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